

Bioaccumulation of Total and Monomethylmercury in Earthworms and the
Ecological Risk to Birds and Mammals at the Northeast Test Hut,
Graces Quarters, Aberdeen Proving Ground, Maryland.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
BAF	Bioaccumulation factor
BTAG	Biological Technical Assistance Group
bw	Body weight
d	Day
DSHE	Directorate of Safety, Health and Environment
EEQ	Environmental effects quotient
EPA	U.S. Environmental Protection Agency
ERA	Ecological risk assessment
K _{ow}	Octanol-water partition coefficient
MDL	Method detection limit
MMHg	Monomethylmercury
PQL	Practical quantitation limit
T-Hg	Total mercury
TRV	Toxicity reference value
USACEHR	U.S. Army Center for Environmental Health Research
wt.	Weight

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EXECUTIVE SUMMARY

Mercury-contaminated soils, which occurred during chemical warfare materiel decontamination studies conducted during the 1950s, were found during the mid 1990s at the Northeast Test Hut, Graces Quarters, Aberdeen Proving Ground, Maryland. The concentrations of total mercury in the soil (0-15 cm horizon; 0-6") ranged from ~0.1 mg/kg dry weight soil (background) up to ~15 mg/kg dry weight. An ecological risk assessment of the mercury contamination, which assumed that all mercury at the site was methylmercury (most toxic form of mercury in soil), indicated that the potential exists for adverse effects to wildlife. Because of the contamination at the site, EPA Region III established a clean up level for total mercury of 0.1 mg/kg dry soil for Graces Quarters. EPA Region III gave the Army the option of conducting bioaccumulation studies with earthworms exposed to contaminated soil at Graces Quarters to determine the bioaccumulation factors (BAFs) for methylmercury which could be used to more accurately assess the risk to wildlife which may feed on earthworms at the site. The current study was initiated to 1) determine the BAFs for total and methylated mercury in earthworms and 2) use the site-specific derived BAFs to re-assess the ecological risk to the American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*) which feed on earthworms exposed to mercury.

Bioaccumulation of Mercury

Three contaminated soil samples, which contained the highest concentration of mercury found at the Northeast Test Hut, an intermediate, and a low concentration of mercury, were used for the bioaccumulation study. A reference soil sample was taken from an uncontaminated site at Graces Quarters. The concentrations of total mercury (T-Hg) in the high, intermediate, low, and reference soils were 11.542, 2.825, 0.156, and 0.085 mg/kg dry weight, respectively. The concentrations of monomethylmercury (MMHg) were 0.00735, 0.00256, 0.00148, and 0.00112 mg/kg dry weight, in the high, intermediate, low, and reference soils, respectively.

Bioaccumulation was determined by the steady state method using the lumbricid earthworm *Eisenia fetida*. The experimental design consisted of a 28-day uptake phase in the three contaminated soils and reference soil followed by a 28-day depuration phase in reference soil only. During the uptake phase, four randomly selected replicates of 10 earthworms/replicate were analyzed for T-Hg and MMHg in each contaminated soil and reference soil at days 1, 2, 4, 7, 14, 21, and 28. Four randomly selected replicates of 10 earthworms/replicate were analyzed for T-Hg and MMHg from each contaminated soil and reference soil at days 35, 42, 49, and 56 during the depuration phase. At each sample period, the earthworms in each replicate were counted (to determine survival), their guts purged for 24 h, and weighed. At the end of the 28-day uptake phase, the earthworms in each remaining replicate were counted, their guts purged for 24 hours, and weighed. The

earthworms were then placed in unused reference soil and sampled at the frequency described above during the 28-day depuration phase. Mercury did not affect survival or growth of the organisms in the four test soils.

The uptake of T-Hg by the earthworm followed first order reaction kinetics in all soil treatments (including the reference soil). A steady state did not occur during the 28-day uptake period. A BIOFAC model analysis predicted the time to 90% steady state to be 38 and 41 days, respectively, in the high and intermediate treatments. The 90% steady state for the earthworms held in the low and reference soil was predicted to be 30 and 36 days, respectively, when all data through day 56 were used as uptake data only. All data through day 56 were used as uptake data because the reference earthworms were exposed for 56 days to a constant concentration of T-Hg and reached a steady state. No difference was found between the low and reference treatments in both the uptake and depuration phase; thus, the data through day 56 were used as uptake data.

The depuration of T-Hg in the high and intermediate treatments followed a two phase elimination model. Depuration initially decreased from day 28 and reached a depuration steady state by day 35. No significant change occurred in T-Hg concentrations at the high and intermediate treatments from days 35 to 56. Total mercury concentrations did not decrease in the low and reference treatments during the depuration phase. The time to 50% clearance was predicted by the BIOFAC model to be 12 days for both the high and intermediate treatment. The model estimated the time to 50% clearance to be 9 and 11 days, respectively, in the low and reference treatments. The BAFs, determined by the ratio of the uptake and depuration rate constants derived by the BIOFAC model for the high and intermediate treatments, were estimated to be 0.7, and 0.6 respectively, when the data through day 35 of depuration were used. The BAFs for the low and reference earthworms were estimated by BIOFAC to be 2.2 and 3.7, respectively, using all data through day 56 as uptake data.

The uptake phase of MMHg followed first order reaction kinetics. As was the case for T-Hg, uptake did not reach a steady state in 28 days. The BIOFAC model predicted the time to 90% steady state to be 172, 192, and 97 days, respectively, in the high, intermediate, and low exposure soils. The steady state estimates have a high degree of variability relative to the steady state estimates for T-Hg. No estimate of time to steady state was made for the earthworms in the reference soil because uptake was continuous and steady state did not occur during the 56-day exposure period.

The depuration of MMHg did not follow a two-phase model as was the case for T-Hg. In the high, intermediate, and low treatments, MMHg initially began to decrease but reversed direction and continued to increase. The reference treatment increased throughout the depuration phase. The times to 50% clearance, which were highly variable, were predicted by BIOFAC to be 52, 99, and 29 days, respectively, in the high, intermediate, and low treatments. The BAFs for the high, intermediate, and low MMHg

treatments were 179, 184, and 232, respectively, using data through day 42 of depuration when net depuration was occurring. The BAF for the reference earthworms was estimated as follows. MMHg bioaccumulation continued to increase throughout the 56-day exposure period. As described above, a steady state occurred in T-Hg bioaccumulation in the reference earthworms. Thus, the worst case assumption was made that the MMHg concentration in the reference earthworms eventually comprised 100% of the T-Hg concentration in the reference earthworms at steady state. The mean T-Hg tissue concentration at steady state was 291 ng/g dry weight tissue. Thus, the assumption was made that the MMHg concentration was 291 ng/g dry weight tissue; thus, the BAF would be 260 (291 ng/g dry weight tissue divided by 1.12 ng/g dry weight soil).

Ecological Risk of Mercury to the Robin and Shrew

The risks to birds and mammals from ingesting mercury-contaminated earthworms and soil in the Graces Quarters environmental risk assessment (ERA) were re-evaluated using the BAFs obtained in the bioaccumulation study. As stated above, the ERA assumed that all mercury in the soil at the Northeast Test Hut was MMHg (most toxic form of mercury in soil) in order to err on the conservative side since adequate bioaccumulation data were not available. Many of the assumptions in the original Graces Quarters ERA regarding exposure scenarios, ecological effects, risk characterization, uncertainties, etc., were used in the re-assessment.

The estimated dietary concentration (i.e., proportion of diet consisting of earthworms as a function of the estimated concentration of MMHg in earthworms) data for the robin and shrew used in the exposure assessment phase of the ERA were re-evaluated as follows. Two scenarios for the proportion of diet consisting of earthworms were taken directly from the ERA. The first highly conservative scenario assumed that 100% of the robin's and shrew's total annual diet was comprised of earthworms. The second less conservative scenario assumed that 22% of the robin's and 31.4% of the shrew's diets were comprised of earthworms. To be conservative, the estimated concentration of MMHg in earthworms was calculated by using the highest BAF (260) obtained in the bioaccumulation study. The concentrations of MMHg in the three soils (high, intermediate, and low) at the Northeast Test Hut and the reference soil were used in the calculations to estimate the concentration of MMHg in earthworms and the soil dose for robins and shrews that inadvertently ingest contaminated surface soil.

The potential exposure concentrations (i.e., total dose, which included both contaminated earthworms and soil) derived in the exposure assessment phase were compared with the original ERA toxicity reference values (TRVs) to calculate the environmental effects quotients (EEQs) used in the risk characterization process. The EEQs for robins from the ingestion of MMHg in earthworms and all surface soils at the Northeast Test Hut were <1 when earthworms were assumed to comprised 22% of a robin's diet. The EEQs for the robin were >1 from the ingestion of MMHg in earthworms

and the high- and intermediate-contaminated soils when it was assumed that earthworms comprised 100% of the robin's diet. The EEQs were <1 from the ingestion of MMHg in earthworms and low-contaminated and reference soils when it was assumed that earthworms comprised 100% of the robin's diet. The less conservative, but more realistic scenario of 22% earthworms in the robin's diet indicates that MMHg at the Northeast Test Hut will not adversely affect robins that ingest earthworms containing MMHg.

The EEQs for shrews from the ingestion of MMHg in earthworms and all surface soils at the Northeast Test Hut were <1 when earthworms were assumed to comprise 100% of a shrew's entire diet and the less conservative 31.4% earthworm diet. Based on these results, it is reasonable to conclude that shrews will not be adversely affected by the ingestion of MMHg in earthworms and surface soils at the Northeast Test Hut.

In summary, no risk was found for robins which have an annual diet that is comprised of 22% contaminated earthworms and soil from any site at the Northeast Test Hut. The highly conservative analysis that assumed a robin's annual diet consisted of 100% contaminated earthworms and soil from the highest and intermediate mercury-contaminated sites at the Northeast Test Hut showed that robins would be at risk. The risk for exposure to contaminated earthworms and soil at the highest mercury concentration detected at the Northeast Test Hut has been eliminated because the soil was removed from the site for use in the bioaccumulation study. Several small surface areas (<1 m²) exist at the site which contain concentrations of mercury similar to the intermediate concentration evaluated in the risk assessment. The areal extent of existing mercury contamination at the Northeast Test Hut is quite small relative to the total area of the Northeast Test Hut. It is orders of magnitude smaller when one includes the entire land mass of Graces Quarters. The uncertainty decreases when one considers that the robin may feed at other uncontaminated sites on Graces Quarters. The current risk analysis showed that it is reasonable to conclude that shrews will not be adversely affected by the ingestion of MMHg in earthworms and surface soils found at the Northeast Test Hut.

1. INTRODUCTION

Mercury-contaminated soil was found in the vicinity of the Northeast Test Hut (Site 12) during the 1993 -1995 Remedial Investigation of Graces Quarters, Aberdeen Proving Ground, Maryland (Figures 1-1, 1-2, and 1-3) (Dames & Moore, Inc., 1997a). Additional sampling in 1997 established the spatial extent of the contamination (Dames & Moore, Inc., 1997b). The contamination of the site appears to have occurred during chemical warfare materiel decontamination studies conducted during the 1950s (Nemeth, 1989). The concentrations of total mercury in the soil (0-15 cm horizon; 0-6") range from ~0.1 mg/kg dry weight soil (background) up to ~15 mg/kg dry weight. An ecological risk assessment of the mercury contamination, which assumed that all mercury at the site was methylmercury (most toxic form of mercury in soil), indicated that the potential exists for adverse effects to wildlife (Dames & Moore, Inc., 1997a). Because of the contamination at the site, EPA Region III established a clean up level for total mercury of 0.1 mg/kg dry weight soil for Graces Quarters (Green, 1998). However, EPA Region III gave the Army the option of conducting bioaccumulation studies with earthworms exposed to contaminated soil at Graces Quarters to determine the bioaccumulation factors for methylmercury which could be used to more accurately assess the risk to wildlife which may feed on earthworms at the site. The current study was initiated to 1) determine the BAFs for total and methylated mercury in earthworms and 2) use the site-specific derived BAFs to re-assess the ecological risk to the American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*) which feed on earthworms exposed to mercury.

Mercury is a naturally occurring element which is ubiquitous in the environment. The element exists in three valence states (0, +1, and +2) as well as in various inorganic and organic complexes. Elemental mercury (Hg^0) is the most common form found in nature. Biogenic emissions to the atmosphere are the most important processes of mercury re-distribution to the environment; anthropogenic emissions (e.g., fossil fuel combustion) account for 10 to 30% of the mercury emitted annually (Stein et al., 1996). The predominant form of mercury in the atmosphere is Hg^0 vapor (95 to 100%) (Munthe, 1994). The ultimate fate of atmospheric mercury is wet and dry deposition, of which the former is probably the most important (Seigneur et al., 1999). Wet deposition can only occur after volatile Hg^0 has been oxidized to water soluble forms, such as divalent mercury (Hg^{2+}) (Munthe, 1994). When deposited to surface soil, mercury is retained primarily as complexes of Hg^{2+} bound with sulfides, clay particles, and organic matter (Keating et al., 1997). Divalent mercury forms strong complexes with organic matter probably through ionic reactions at naturally occurring sulfhydryl binding sites (Loux, 1998).

Divalent mercury can be oxidized in soil by biotic or abiotic reactions. When mercury is oxidized to Hg^{2+} , it can be methylated by anaerobic, and to a lesser extent, aerobic microorganisms to form primarily monomethylmercury (CH_3Hg^+). Dimethylmercury

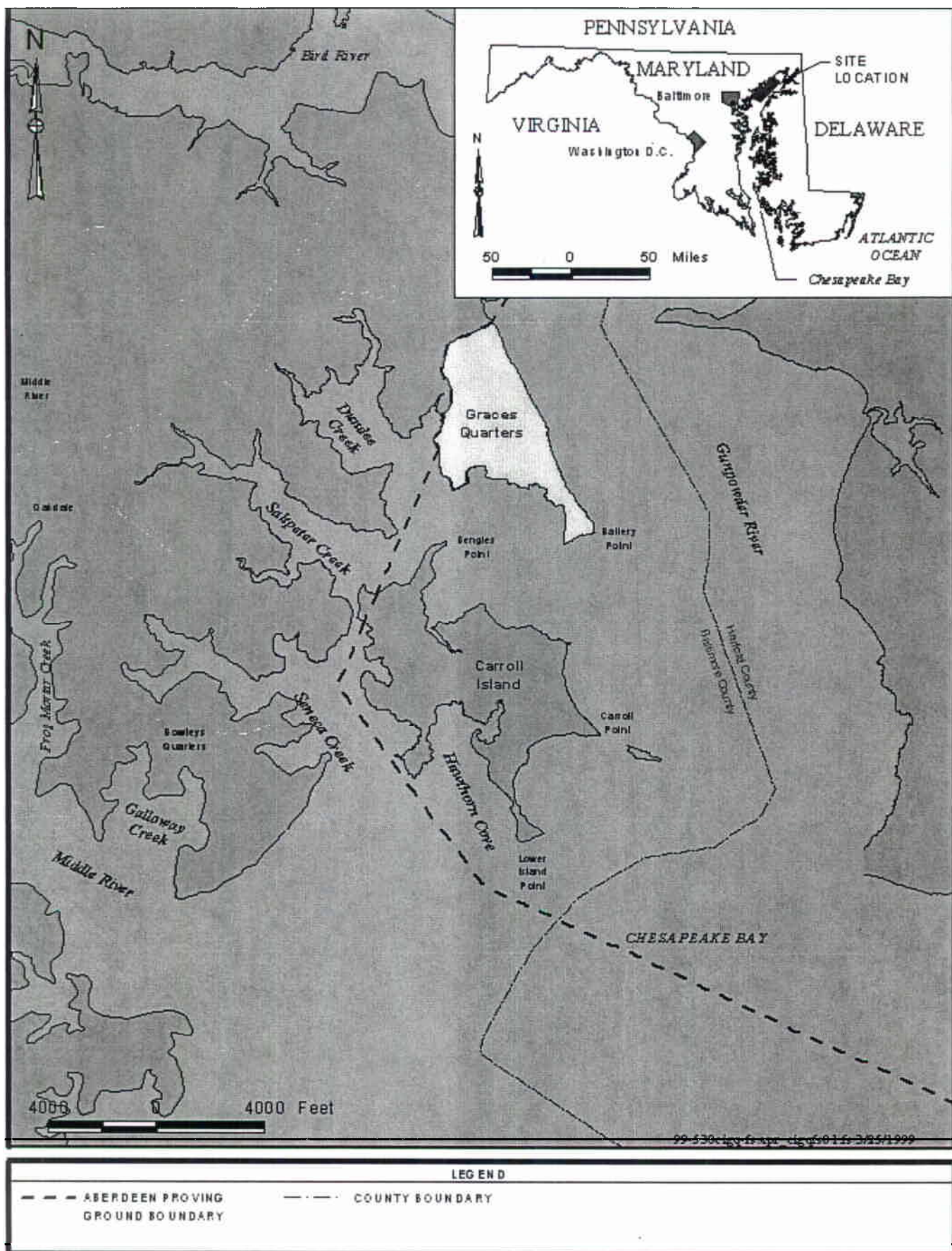


Figure 1-1. Graces Quarters - Location Map

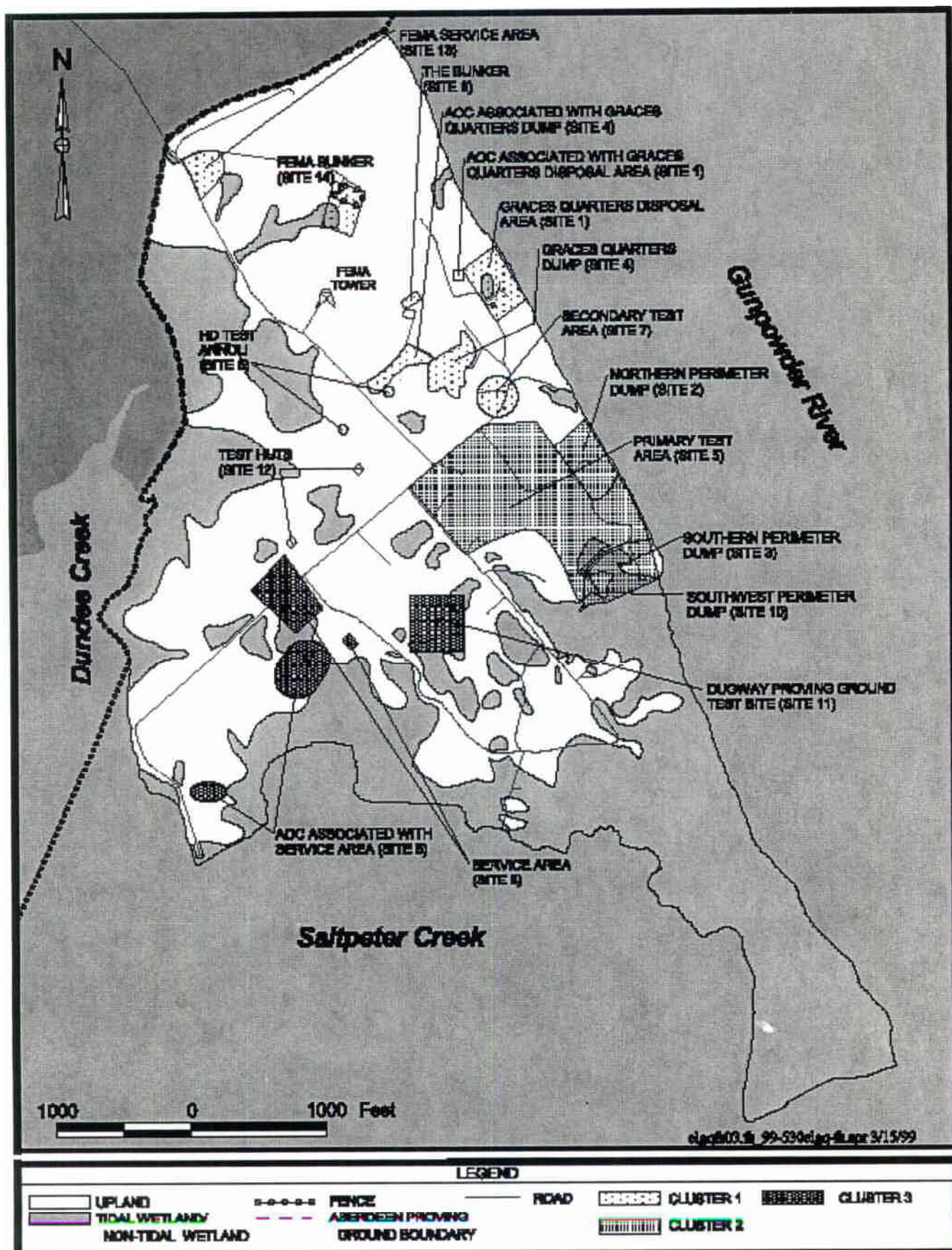


Figure 1-2.

Graces Quarters - Cluster and Site Locations

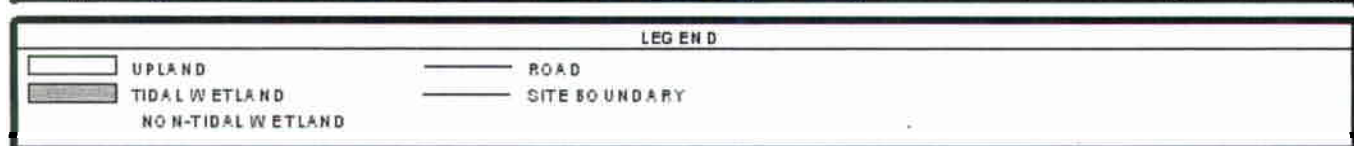


Figure 1-3. Graces Quarters - Test Huts (Site 12) Location

$[(\text{CH}_3)_2\text{Hg}]$ formation, which is more common in marine sediments, can also occur and accounts for approximately 3% of the methylmercury in marine sediments (Loux, 1998; Stein et al., 1996). Monomethylmercury formation is favored under acidic conditions; $(\text{CH}_3)_2\text{Hg}$ formation is favored under neutral or alkaline conditions in the presence of a strong complexing agent (Stein et al., 1996). The amount of methylmercury in soils is low relative to total mercury. According to Boudou and Ribeyre (1997), the average percentage of methylmercury to total mercury in soils ranges between 0.5 and 1.5%.

Elemental and inorganic mercury in general are less toxic to terrestrial organisms than methylmercury (Heaton et al., 1998; Stein et al., 1996). Likewise, metallic and inorganic mercury do not bioaccumulate in terrestrial organisms to the degree that methylmercury does (Boudou and Ribeyre, 1997). Bioaccumulation factors (BAFs) for total mercury in earthworms, which are important in the diets of vermivorous wildlife, are usually 1 or less (no bioaccumulation); however, uptake factors up to ~10 have been reported (Cocking et al., 1994; Fischer and Koszorus, 1992; Sample et al., 1999). Limited studies of food chain transfer of total mercury from contaminated surface soil to small mammals which consume earthworms as part of their diet indicate that mercury concentrations in biota do not exceed concentrations in the soil (Bull et al., 1977; Talmage and Walton, 1993). In contrast to inorganic mercury, a number of studies have shown that methylmercury can bioaccumulate in birds and mammals, particularly in piscivorous wildlife (Wolfe et al., 1998). With the exception of a study by Beyer et al. (1985) who demonstrated that methylmercury can bioaccumulate in earthworms, little information is available concerning the bioaccumulation of methylmercury in earthworms exposed to mercury-contaminated soils. Because of the importance of earthworms in many temperate terrestrial ecosystems as a food source for small birds and mammals, this study was initiated to determine the bioaccumulation and depuration of total mercury and monomethylmercury for an earthworm exposed to Hg-contaminated soil. The bioaccumulation of total mercury and monomethylmercury is presented in Section 2 of this report. The ecological risk to the American robin and short-tailed shrew which feed on earthworms exposed to mercury is presented in Section 3.

2. BIOACCUMULATION OF MERCURY

2.1 Materials and Methods

2.1.1 Study Soils

Three contaminated soil samples and one reference soil were used in the study. The contaminated soil samples were taken in the vicinity of the Northeast Test Hut. Figure 2-1 shows the location and total mercury concentration ($\mu\text{g/g}$ dry weight soil) of all soil samples in the 0-15 cm (0-6") horizon taken during the 1997 survey to establish the spatial extent of contamination in the area (Dames & Moore, Inc., 1997b). The location of the three sites from which soil was taken for the study are labeled high, intermediate, and low. The Aberdeen Proving Ground sample identification numbers for the high, intermediate, and low mercury soil sample sites are TQ1245D, TQ1227, and TQ1246, respectively. The reference soil sample was taken approximately 120 m (~400') east of the Graces Quarters entrance gate approximately 15 m (~50') inside the boundary fence.

The three contaminated and reference soils were all Mattapeake/Mattapex soils. Approximately 30 kg wet weight soil (~65 pounds wet weight) were taken via shovel and/or hand trowel at each Northeast Test Hut sample site on April 16, 1998; ~45 kg wet weight soil (~100 pounds wet weight) were taken from the reference site. One composite sample was taken at each site. Each soil sample was placed in a 4 mil plastic bag and tapped closed which was in turn placed in a second 4 mil bag and tapped closed. All samples were placed in individual ice chests and kept cold (ice) in the field and during transport back to laboratory. All samples were stored in their original containers in the dark at 4°C in the laboratory. The exposure phase of the study was initiated one week after the soil samples were collected.

The soil samples were prepared for testing via the procedures outlined in the ASTM standard guide for conducting laboratory soil bioaccumulation tests with lumbricid earthworms (ASTM, 1998). Briefly, all indigenous earthworms, cocoons, insects, and other debris were removed from the soils before the soils were sieved through a 6.35 mm (0.25") stainless steel sieve and homogenized. The four soils were characterized for pH, percent organic matter, cation exchange capacity, total nitrogen, particle size distribution (percent sand, silt, and clay), and percent water content. The soils were also analyzed for metals (including monomethylmercury), volatile organics, base neutrals, acid compounds, pesticides/PCBs, herbicides, and explosives. The various chemical methods used to analyze the above parameters are given in Appendix A. Before the earthworms were placed in the soils, water content was adjusted to ~45% moisture content and pH was adjusted to ~6.

2.1.2 Test Organisms

The lumbricid earthworm *Eisenia fetida* was used for all bioaccumulation tests. The earthworms were cultured in-house by the procedures given in ASTM (1998). Briefly, *E. fetida* was reared in a bedding of sphagnum peat moss with the pH adjusted to approximately 7 using calcium carbonate hydrated with reverse osmosis water. Moisture content was monitored on a weekly basis. Covered plastic trays were maintained so that there was no standing water in the bottom of the tray and the surface of the bedding was not dry. The trays were held under continuous lighting (~40 foot candles) at 22°C (± 1°C). The animals were fed fermented alfalfa pellets once or twice per week, depending on the number of individuals in a tray. The culture carrying capacity recommended in ASTM (1998) was followed. The bedding was periodically changed to prevent overcrowding. The bioaccumulation tests were initiated with sexually mature, fully clitellate adults.

In addition to the earthworms used in the exposure phase, several clitellate earthworms were taken from the high contaminated soil only on the day the soil was obtained in the field. No attempt was made to identify the earthworms or standardize the size of the organisms. The earthworms were held in soil at ambient temperatures (~20 °C) while being transported back to the laboratory. The guts of the earthworms were purged for 24 h as described below. Sufficient earthworms for three replicates were shipped overnight as described below for total mercury (T-Hg) and monomethylmercury (MMHg) analysis.

2.1.3 Experimental Procedures

Bioaccumulation was determined by the steady state method. The experimental design consisted of a 28-d uptake phase in the three contaminated soils and reference soil followed by a 28-d depuration phase in reference soil only. During the uptake phase, four randomly selected replicates of 10 earthworms/replicate were analyzed for T-Hg and MMHg in each contaminated soil and reference soil at days 1, 2, 4, 7, 14, 21, and 28. Four randomly selected replicates of 10 earthworms/replicate were analyzed for T-Hg and MMHg from each contaminated soil and reference soil at days 35, 42, 49, and 56 during the depuration phase. The day 28 data were also used as day 0 for the depuration statistical analysis (Sect. 2.1.5). At each sample period, the earthworms in each replicate were counted (to determine survival), their guts purged for 24 hours, and weighed as described below. At the end of the 28-day uptake phase, the earthworms in each remaining replicate were counted, their guts purged for 24 hours, and weighed. The earthworms were then placed in unused reference soil (soil replicates prepared from the original reference soil two days before the earthworms were transferred) and sampled at the frequency described above during the 28-day depuration phase. Upon transfer to the reference soil, all earthworms were observed burrowing into the surface with no observable differences between earthworms in the treatments.

Three days prior to earthworms being placed in the exposure soils, all earthworms were placed in homogenized reference soil (moisture content and pH adjusted as described above) to “acclimate” to the Mattapeake/Mattapex soil matrix. Two days before the exposures were initiated, 56 replicates of 400 g of soil/replicate of each soil type (three contaminated and one reference soil) were loaded into 473 mL glass enclosed containers fitted with Teflon® -lined screw-on lids pierced with a hole for ventilation. Twenty-four hours prior to the start of the exposure, all study earthworms including the reference earthworms were removed from the reference soil and randomly placed in groups of 10 in polystyrene petri dishes (20 x 100 mm) lined with moist filter paper to purge their gut contents. After purging, each group of 10 earthworms was rinsed in reverse osmosis water, blotted gently by placing between layers of lint-free paper towels and weighed. Forty-four replicates of 10 earthworms/replicate were randomly loaded into each soil type (day 0 of the study). All earthworms were observed burrowing into the soil surface with no observable differences between treatments. All test vessels were randomly placed in a walk-in environmental chamber maintained at 22 °C (\pm 0.2 °C) with continuous lighting of approximately 40 foot candles at the surface of the soil. All “acclimation” trays and purging dishes were also placed in the walk-in environmental chamber maintained at 22 °C (\pm 0.2 °C) with continuous lighting of approximately 40 foot candles at the surface of the soil and filter paper, respectively.

At day 0 of the study, four replicates of soil from each of the three experimental soils and reference soil were randomly taken for T-Hg and MMHg analyses. Four replicates of 10 earthworms/replicate were also randomly selected at day 0 for T-Hg and MMHg analyses in the reference soil only. In addition, four test vessels per soil type containing only soil were randomly placed in the environmental chamber for T-Hg and MMHg analyses at day 28 of the uptake phase.

During the 56-day test, soil temperature and percent moisture were monitored two times per week in a composite of four replicates from each of the four test soils. Soil moisture was maintained at approximately 45% of water holding capacity (van Gestel et al., 1992). Soil moisture was adjusted if necessary in all remaining replicates by reverse osmosis water. The earthworms were fed weekly during the uptake and depuration phases of the study (Gibbs et al., 1996). A bolus of fermented alfalfa was added to a hole in the soil of the test vessel of each replicate at a rate of 350 mg/g earthworm/week as recommended by van Gestel et al. (1992). Excess food was removed after two days to prevent fungal growth. Soil pH was measured at the beginning of both the uptake and depuration phase in a composite of four replicates from each soil type.

2.1.4 Mercury Analyses

The earthworms in each replicate were combined for chemical analyses. Each replicate was analyzed individually. Total mercury and MMHg analyses were made on the whole animal. After the earthworms in each replicate were purged for 24 hours and

weighed, they were placed in acid rinsed 40 mL glass vials with Teflon®-lined lids. The vials were refrigerated at 4 °C prior to being packed with blue ice and shipped overnight in polyfoam-lined containers to Brooks Rand, Ltd. (Seattle, Washington) for analysis. All soil samples (~10 g/replicate) were also placed in acid rinsed 40 mL glass vials with Teflon®-lined lids and treated in the same manner as the earthworms.

Total mercury in both the earthworm and soil samples was determined by cold vapor atomic fluorescence spectrophotometry (Brooks Rand, Ltd., 1998a). Briefly, the solid samples (both earthworm and soil) were digested with a 70:30 nitric:sulfuric acid solution and further oxidized with bromine chloride. The oxidation/digestion procedure converted all mercury species to Hg^{2+} . The samples were then reduced by tin chloride to form volatile Hg^0 (elemental mercury). The samples were purged with Hg-free nitrogen and the mercury collected and concentrated on a gold trap. The gold trap was then heated, thermally desorbing the mercury, which was swept by an inert carrier gas through an atomic fluorescence mercury detector. Peak area (fluorescence response) was measured (as elemental mercury) using a standard calibration curve.

Monomethylmercury was also determined by cold vapor atomic fluorescence spectrophotometry (Brooks Rand, Ltd., 1998b). The earthworms were digested in a potassium hydroxide/methanol solution. The soil samples were distilled in Teflon® distillation equipment. All samples were then ethylated forming a methyl-ethyl mercury derivative. The derivative was then purged onto a precollection trap. The trap was moderately heated under the flow of an inert carrier gas, releasing the mercury species. The mercury species were then separated using gas chromatography, after which the species were pyrolytically broken down to Hg^0 prior to passing through an atomic fluorescence mercury detector. Peak area (fluorescence response) was measured (as elemental mercury) using a standard calibration curve.

The method detection limit (MDL) and practical quantitation limit (PQL) using the above analyses for T-Hg in both tissue and soil for a 5 g sample volume (wet weight) are both 0.1 ng/g dry weight. The MDL and PQL for MMHg in tissue are 1 and 5 ng/g (ppb), respectively. The MDL and PQL for MMHg in soil are 0.002 and 0.01 ng/g, respectively. Smaller sample volumes could be used; however, the resulting detection limits would be higher if <1 g was available. Tissue and soil dry weight were determined by gravimetrically (Brooks Rand, Ltd., 1998c).

2.1.5 Data Analyses

The bioaccumulation data in this study were initially treated as though the earthworms were a one-compartment model. We are aware that the volume in a one-compartment model should be constant (i.e., no growth); however, it is difficult to conduct assays where no growth occurs. Growth can be modeled and subsequently corrected but a number of assumptions must be made when growth is corrected via modeling. Although

the data were not corrected for growth, the conceptual framework for a one-compartment model can be used to explain the trends found in the data obtained in this study. In a one-compartment model, the uptake of a chemical is assumed to be directly proportional to the exposure concentration in the soil (Spacie and Hamelink, 1995). Likewise, the rate of depuration is assumed to be directly proportional to the concentration in the animal.

The bioaccumulation of T-Hg and MMHg in the earthworms was estimated as described below by the BIOFAC model which treats each organism as a one-compartment model (Blau and Agin, 1978). BIOFAC consists of a nonlinear parameter estimation routine which generates optimal estimates of the uptake (k_1) and depuration (k_2) rate constants from a set of sequential time-concentration data. The data are weighted by a normality preserving transformation to reflect any time- or concentration-related trends in variability. Uncertainty in the parameters as well as the validity of the model are also estimated by the model. The uptake and depuration rate constants were used to calculate the bioaccumulation factor (BAF) of each material as described below. In addition to the uptake and depuration rate constants and the BAFs, BIOFAC was also used to estimate time to reach 90% steady state and 50% clearance of the chemicals.

BIOFAC was used in two scenarios to estimate the above parameters for T-Hg in the high and intermediate treatments. A basic assumption of a one compartment bioaccumulation study is that the organism will be placed in uncontaminated media during the depuration phase to determine the depuration rate kinetics. As discussed below, the reference soil also contained mercury (both T-Hg and MMHg) and as a consequence the earthworms were continually exposed to a low level of mercury during the depuration phase of the study. As will be shown below, a steady state occurred in the high and intermediate T-Hg treatments after an initial elimination of T-Hg occurred in the depuration phase. To avoid confusion, we will refer to the steady state that occurred after the initial elimination of mercury in the depuration phase as the depuration steady state to distinguish it from the "normal" steady state that occurs from the uptake of a material.

The BIOFAC model can estimate the depuration rate constant (k_2) and time to 50% elimination directly from the uptake phase of a study without having a depuration component if a steady state occurs. Depuration kinetic data are more rigorous, however, if a good initial estimate of depuration is available (Blau and Agin, 1978). Total mercury concentrations in the high and intermediate treatments initially decreased from day 28 but reached a depuration steady state by day 35 during the depuration phase (Sect. 2.2.3). Thus, BIOFAC was used to estimate the above parameters in the high and intermediate T-Hg treatments by using all of the 28-day uptake data and depuration data through day 35 where net elimination of T-Hg occurred. No elimination of T-Hg occurred in the low and reference treatments; however, a steady state (uptake) of T-Hg uptake was reached during the depuration phase (see below). Thus, the BIOFAC estimates for the low and reference treatments were made using all data through day 56 as uptake data only. Bioaccumulation factors were also estimated for the low and reference treatments by using the average

uptake steady state T-Hg concentrations in the tissues from day 28 to day 56 of depuration divided by the average T-Hg concentrations in the soil (see below).

The BIOFAC analysis of the MMHg data was performed as follows. The uptake of MMHg in all treatments followed first order rate kinetics; however, a steady state did not occur during the uptake phase. During depuration, MMHg initially started to decline through day 42 of depuration, followed by a continuation of uptake in the high, intermediate, and low treatments (see below). Thus, BIOFAC was used to estimate the above parameters by using all the 28-day uptake data and depuration data through day 42. In the reference earthworms, MMHg concentrations continued to increase at the same rate as those in the uptake phase (earthworms were held in the same concentration of MMHg for the entire 56 days of exposure) during the depuration phase. Since no steady state or measurable depuration occurred in the reference MMHg-exposed earthworms, the BIOFAC model was not used to estimate the BAF and associated kinetic data. As discussed in Section 2.2.4, the BAF was estimated by making the worst case assumption that the T-Hg concentration in the reference earthworms at steady state was 100% MMHg.

To test the assumption that the shape of the curves was the same among treatments, a model was constructed for the logarithm of mercury concentration (both T-Hg and MMHg) using linear and quadratic terms to form a low order polynomial of the logarithm of concentration (SAS, 1989). The model for mercury concentration at each time period (day) and for each soil treatment was:

$$\text{Mercury} = e^{(\alpha_{ij} + \beta_{1ij}\text{day} + \beta_2\text{day}^2)}$$

where:

$$\begin{aligned} i &= \text{ordinal number for treatment;} \\ j &= \text{ordinal number for period; and} \\ \alpha, \beta_1, \text{ and } \beta_2 &= \text{model parameters.} \end{aligned}$$

A variable (pday) was created for the within phase day. For the uptake phase (day 0 to day 28) the variable was the same as day; for the depuration phase (day 28 to day 56) it was equal to day -28. The variable was then scaled within each concentration and phase (uptake and depuration) to have mean zero before squaring the term for use in the polynomial regression. As a result of the scaling manipulation, the estimates of the linear and quadratic coefficients for the polynomial expression were independent. The linear coefficient was interpreted as the derivative of the curve at the midpoint of the phase. Thus, it represented the average change in mercury concentration for the phase. The quadratic term yielded information about whether the change in uptake was accelerating or decelerating with time. The first step was to test for consistency of the polynomial coefficients over the treatment conditions. Because it was assumed that the rates would differ between the uptake and depuration phases, the tests were performed separately by

phase.

In both the uptake and depuration phase at all treatments, the logarithms of T-Hg had curvature with respect to time. In the uptake phase, the curvature was negative with respect to time which indicates that the rate of accumulation slowed. The degree of curvature was not significantly different for T-Hg ($p < 0.6189$) or MMHg ($p < 0.7008$). In the depuration phase, the interaction statistics for the quadratic terms showed that the curvature of the four treatments differed significantly for both T-Hg ($p < 0.0005$) and MMHg ($p < 0.0279$). Thus, the statistical model for both the T-Hg and MMHg uptake data was set to use only one parameter of curvature (assuming equal curvature among treatment conditions). Four estimates of curvature were used for both the T-Hg and MMHg depuration analyses.

Two MMHg tissues values were less than the detection limit on day 1 in the intermediate concentration (Appendix C; Table C-2). To maintain equal sample sizes, the two values were replaced by one-half the detection limit before performing the statistical analysis.

The effect of mercury on growth (dry weight) within the uptake and depuration phases was analyzed after the following was considered. A handling effect appears to have occurred at the beginning of the uptake and depuration phase while clearing soil from the earthworm's guts (animals were not feed for 24 h) before weighing the organisms (Fig. 2-2). The handling effect resulted in a reduction of weight at the beginning of both the uptake and depuration phases. Thus, the first three growth observations (days 1, 2, and 4) of the uptake period were omitted from the analyses. The depuration analyses were run on data from days 35 through 56.

The effect of mercury on growth, which was fairly linear in both the uptake and depuration phases, was analyzed by analysis of covariance using the following model (SAS, 1989):

$$\text{Growth} = m + a_i + b_j + (g_{ij})(t) + e_{i,j,t,k}$$

where:

m	=	overall mean;
a_i	=	mean effect of mercury;
b_j	=	mean effect of phase (uptake vs depuration);
g_{ij}	=	growth rate for each mercury treatment and phase; and
$e_{i,j,t,k}$	=	error deviation for treatment i , phase j , time t , and replicate k .

Growth hypotheses were tested by comparing slopes and intercepts among treatments.

The model of covariance was first run to test the null hypothesis that no study variables had an effect on growth. Using growth (dry weight) as the dependent variable, the following independent variables were initially tested:

Concentration	=	high, intermediate, low, and reference concentrations;
Phase	=	uptake phase and depuration phase; and
Pday	=	day within a phase.

For each phase (uptake and depuration), pday was set at 0 at the beginning of the phase. A two-way interaction between treatment concentration and phase and a three-way interaction between day within phase, treatment concentration, and phase were not significant and were dropped from further consideration (Sect. 2.2.2). A second model run was made omitting the interactions which were not significant.

The statistical analysis of the effect of mercury on survival during the study was problematic in that little mortality occurred. Because some treatment groups had zero mortality with no variation, the homogenous variance assumption of analysis of variance (ANOVA) is violated. Maximum likelihood methods such as logit and probit analyses perform poorly when parameters are at or near the edge of the parameter space. In the present study, survival is near the upper bounds of 100%. Nonparametric methods do not perform well in the presence of many ties that will result from the many replicates with 100% survival. Given the above concerns and the fact that survival in all four treatments met the ASTM (1998) test acceptability criteria of >90% survival for the reference animals, the data were not analyzed statistically (see Sect. 2.2.2).

2.2. Results and Discussion

2.2.1 Chemical Characteristics of the Soils

The chemical characteristics, chemical methods, and reporting limits for the four soils measured prior to the start of the study are given in Appendix A. The general chemical characteristics and metals in the four soils measured prior to the start of the study are summarized in Tables 2-1 and 2-2. Lead, mercury (total), and zinc occurred above EPA Region III BTAG screening levels of 46.7, 0.15, and 150 mg/kg dry weight, respectively, in the high and intermediate soils (U.S. EPA, 1995). In addition to the three metals which exceeded BTAG's screening level values, aluminum, antimony, copper, manganese, and selenium, also exceeded NOAA's screening level values in one or more of the four soils (Buckman, 1999). NOAA's screening levels differ from BTAG's levels in that NOAA's screening levels are the geometric means of the concentrations found in natural soils in the United States whereas BTAG's screening levels are based on toxicological data. With the exception of bis (2-ethylhexyl) phthalate in the high and intermediate soils, no other base neutral priority pollutants were found in the soils (Appendix A, Table A-4). No priority pollutant volatile organics, acid extractables,

organophosphorous pesticides, chlorinated pesticides and herbicides, or nitroaromatic and nitramine explosives were found in the soils at the reporting limits given in Appendix A.

Table 2-1. Summary of General Chemical Characteristics of the Exposure Soils Before pH and Water Content Were Adjusted for the Assays^a

Analyte	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
Ammonia (as N)	15.8	7.6	5.9	6.4
Cation Exchange Capacity	17.3	16.4	17.1	13.8
Grain Size:				
Clay (%)	14.6	13.2	12.2	6.7
Silt (%)	39.8	42.0	41.0	43.2
Fine Sand (%)	39.1	36.6	41.6	45.6
Medium Sand (%)	5.9	4.2	4.9	4.3
Course Sand (%)	0.6	4.0	0.3	0.2
Moisture (%)	24.5	22.2	24.6	26.2
Nitrate + Nitrate (as N)	13.3	10.8	10.2	13.1
pH	7.52	7.09	5.86	4.40
Total Kjeldahl Nitrogen	396	206	233	597
Total Organic Carbon	31800	23300	26900	42400
Total Solids (%)	75.5	76.3	75.7	74.0

^a All units are in mg/kg dry weight soil except for cation exchange capacity (meq/100g), grain size (%), moisture (%), pH (standard units), and total solids (%).

Table 2-2. Summary of Metals in the Exposure Soils^a

Analyte	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
Aluminum (Al)	8300	1000	8700	5200
Antimony (Sb)	0.6	0.5	0.4	1.0
Arsenic (As)	3.6	4.0	3.5	2.1
Barium (Ba)	67	160	55	14
Beryllium (Be)	0.5	0.6	0.4	0.2
Cadmium (Cd)	0.48	0.35	0.13	0.6
Calcium (Ca)	860	5400	1500	520
Chromium (Cr)	13	11	8.2	5.9
Cobalt (Co)	4.5	5.0	3.6	2.9
Copper (Cu)	23	11	8.4	5.2
Iron (Fe)	8600	9700	7700	7600
Lead (Pb)	93	110	25	28
Magnesium (Mg)	1100	1800	940	450
Manganese (Mn)	380	590	340	130
Mercury; T-Hg (Hg ⁺²)	10.086	2.685	0.149	0.076
Mercury; MMHg (CH ₃ Hg ⁺)	0.00518	0.00206	0.00099	0.00054
Nickel (Ni)	9.4	9.3	6.6	5.2
Potassium (K)	470	460	420	320
Selenium (Se)	0.4	0.4	0.3	<0.25
Silver (Ag)	0.07	0.08	0.05	0.28
Sodium (Na)	390	500	400	420
Thallium (Tl)	0.1	0.1	0.1	<0.1
Vanadium (V)	17	19	14	15
Zinc (Zn)	160	210	59	34

^a All units are in mg/kg dry weight soil.

The mean temperature, moisture, and pH range, during uptake and depuration in the four study soils were 21.9 °C, 47%, and 5.5-6.0, respectively (Appendix B). The average concentration of T-Hg in the high, intermediate, low, and reference soils (mean of 4 replicates at day 0 and 4 replicates at day 28) was 11,542, 2,825, 156, and 85 ng/g dry weight soil, respectively. The average concentration of MMHg in the high, intermediate, low, and reference soils (mean of 4 replicates at day 0 and 4 replicates at day 28) was 7.35, 2.56, 1.48, and 1.12 ng/g dry weight soil, respectively. The concentrations of T-Hg and MMHg in each replicate at days 0 and 28 for the high, intermediate, low, and reference soils are given in Appendix B, Tables B1- through B-4. The concentration of T-Hg and MMHg in the peat moss used to culture the earthworms was 35.458 and 0.420 ng/g dry weight peat moss, respectively.

2.2.2 Survival and Growth

The survival data for the earthworms exposed to the treatment soils and reference soil are summarized in Appendix C. Percent survival of all replicates combined at the end of the 56-day exposure in the high, intermediate, low, and reference soils was 94.6, 99.0, 98.5, and 99.6%, respectively. As discussed in Section 2.1.5, the statistical analysis of the data was problematic in that little mortality occurred and thus, the assumptions of both parametric and nonparametric statistics are violated. Given the reservations presented in Section 2.1.5 and the fact that survival in all four treatments was greater than the test acceptability criteria of >90% survival in the reference animals (ASTM, 1998), the data were not treated statistically.

A few data are available on the toxicity of T-Hg to earthworms. Fisher and Koszorus (1992) exposed adult *E. fetida* to 100, 250, and 500 mg/kg T-Hg (wet weight soil) for eight weeks and found 0, 25, and 100% mortality, respectively. We found mortality that ranged from 0.4% in the reference earthworms up to 5.4% in the earthworms exposed to 11.542 mg/kg T-Hg (dry weight) for eight weeks. Neuhauser et al. (1980) held *E. fetida* in various manures (no detectable mercury present) for 36 weeks and found mortality rates of 9 to 15% at the end of 36 weeks. If one assumes that mortality was distributed equally during the 36-week period, then mortality at eight weeks would range from 2.8 to 3.3%. Cocking et al. (1994) exposed the adult earthworm *Lumbricus terrestris* for 30 days to a series of T-Hg concentrations which ranged from background (<0.4 mg/kg soil dry weight) to 50 mg/kg soil dry weight. The average mortality rate was 16.2%; mortality was not correlated with mercury concentration. Abbasi and Soni (1983) reported a 30-d LC50 of 1.51 mg Hg²⁺/kg soil (wet weight?) for the adult earthworm *Octochaetus pattoni*. In the current study, 2.5% of the adult *E. fetida* held for 28 days at 11.542 mg/kg (dry weight soil) died; no mortality occurred in 28 days at 2.825 mg/Kg (dry weight soil). The 30-d LC50 of Abbasi and Soni (1983) is much lower than the mortality rates found for *E. fetida* and *L. terrestris*. The data of Abbasi and Soni (1983) suggest that *O. pattoni* is more sensitive to mercury than *E. fetida* and *L. terrestris* over comparable exposure periods.

The toxicity of MMHg to earthworms has not been systematically studied. In a study of the accumulation of methylmercury and its effect on regeneration in adult *E. fetida*, Beyer et al. (1985) exposed the earthworms for periods of 6-12 weeks to soil containing 0, 1, 5, 25, and 125 mg/kg MMHg (wet weight) measured as T-Hg. All earthworms exposed to 25 and 125 mg/kg (wet weight) died in the 6-week exposure period. After a 12-week exposure to MMHg, survival rates of 97, 92, and 79% were observed for earthworms held in 0, 1, and 5 mg/kg (wet weight) soils. All surviving earthworms in the 0 and 1 mg/kg (wet weight) group regenerated segments; 71% of the earthworms in the 12-week exposure to 5 mg/kg (wet weight) regenerated. The concentration of MMHg in the high treatment soil (0.007 mg/kg dry weight) in the present study is orders of magnitude lower than the concentrations which affected survival and segment regeneration in the Beyer et al. (1985) study.

The average growth of the earthworms at each sample period during uptake and depuration in the high, intermediate, low, and reference soil is shown Figure 2-2. The value at each sample period is the mean of four replicates. The growth data for each replicate are given in Appendix C. As discussed in Section 2.1.5, a handling effect appears to have occurred at the beginning of the uptake and depuration phase which resulted in a reduction of weight at the beginning of both the uptake and depuration phases. Thus, growth from day 7 to day 28 and day 35 to day 56 was used for the uptake and depuration analyses, respectively.

A model of covariance was used to test the null hypothesis that no study variables had an effect on growth (Sect. 2.1.5). An initial model run showed that one or more independent variables had a significant ($p < 0.0001$) effect on growth. As shown in Appendix D, Table D-1, five terms were found to be important; two interaction terms were not significant. Thus, a second model run was made omitting the two interactions which were not significant. The following terms, which are summarized in Appendix D, Table D-2, were found to be important. Significant differences were found between the uptake and depuration phase ($p < 0.0003$), day within a phase ($p < 0.0001$), two-way interaction between day within a phase and concentration ($p < 0.0343$) and two-way interaction between day within a phase and uptake and depuration phase ($p < 0.0062$). No difference was found between concentrations ($p < 0.2304$). The significance of the two-way interaction of day within a phase versus the uptake and depuration phase ($p < 0.0062$) indicates that growth rates differed between the uptake and depuration phases. A test of the hypothesis that growth rates in the uptake phase equaled growth rates in the depuration phase showed that growth was significantly ($p < 0.0062$) greater in the depuration phase.

Growth was linear in both the uptake and depuration phases in all exposures when growth from day 7 to day 28 in the uptake phase and day 35 to day 56 in the depuration phase were considered (Fig. 2-2). Several studies have shown that the growth of *E. fetida* is linear (as measured by an increase in weight) for several weeks following sexual maturity

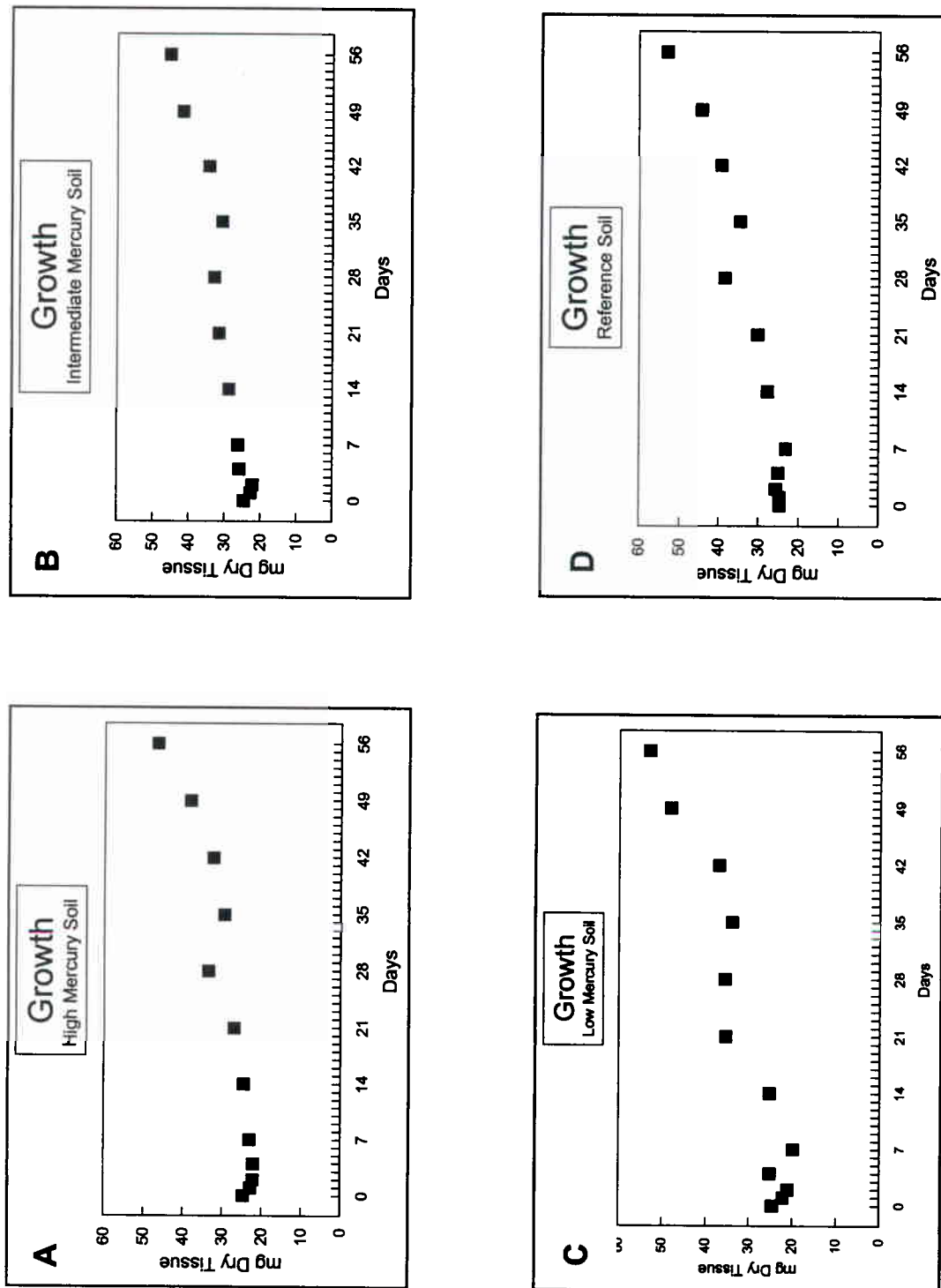


Figure 2-2. Growth of Earthworms Exposed to the High (A), Intermediate (B), Low (C), and Reference (D) Soils. Legend: Squares- Mean of Four Replicates.

(Hartenstein et al., 1979; Jefferies and Audsley, 1988; Neuhauser et al., 1980). The duration of the linear or rapid growth phase has been shown to be a function of the growth medium (Hartenstein et al., 1979 and Neuhauser et al., 1980).

The average growth rate of the reference earthworms was 5.1 mg/week dry weight in the uptake phase from day 7 to day 28. The average growth rate was 6.1 mg/week dry weight in the depuration phase from day 35 to day 56. The average growth rates of the earthworms in the current study, which were grown in Mattapeake/Mattapex soil, are lower than the rates reported in other studies for *E. fetida* where the growth medium was one of several manures. The growth rate for *E. fetida* in soil overlaid with horse manure (5 parts manure:1 part soil) at a similar stage of development and population density (8 earthworms in 300 g media) to that used in this study (10 earthworms in 400 g soil) was ~12.8 mg/week dry weight (Neuhauser et al., 1980). The growth rate for *E. fetida* grown in pig manure was ~8.3 mg/week dry weight (Jefferies and Audsley, 1988). Growth rate data in the above studies by Neuhauser et al. (1980) and Jefferies and Audsley (1988) were given in wet weight and subsequently corrected to dry weight by using a factor of 0.1 (*E. fetida* dry weight is ~ 10% of wet weight; Gibbs et al., 1996). The difference in growth rates between the studies may be attributable to different growth media; lower weights in the present study because gut contents were voided prior to weighting (gut contents were not cleared in the other studies); and possibly growth temperatures in the Neuhauser et al. (1980) study which were 3 °C higher than the current study.

In the current study, no difference in growth rate was found between the three treatments and the control earthworms in the uptake phase. Likewise, no difference in growth rate was found between the three treatments and the control earthworms in the depuration phase. Growth was found to be significantly higher in the depuration phase relative to the uptake phase. It is not clear why growth rates in the present study were higher during the depuration phase particularly when one considers that the reference and low exposure groups were held in essentially the same concentrations of mercury in both the uptake and depuration phases.

2.2.3 Bioaccumulation of Total Mercury

The uptake and depuration of T-Hg by the earthworm in the high, intermediate, low, and reference soils are shown graphically in Figure 2-3, Panels A-D, respectively. The raw data for T-Hg in the tissue of the earthworms exposed to the study soils are given in Appendix C. The uptake of T-Hg at the high, intermediate, and low/reference treatments followed first order reaction kinetics. As discussed below, no difference was found between the low and reference treatments. This is most likely due to the small differences in mercury concentrations between the low and reference soils relative the higher mercury concentrations in the high and intermediate soils.

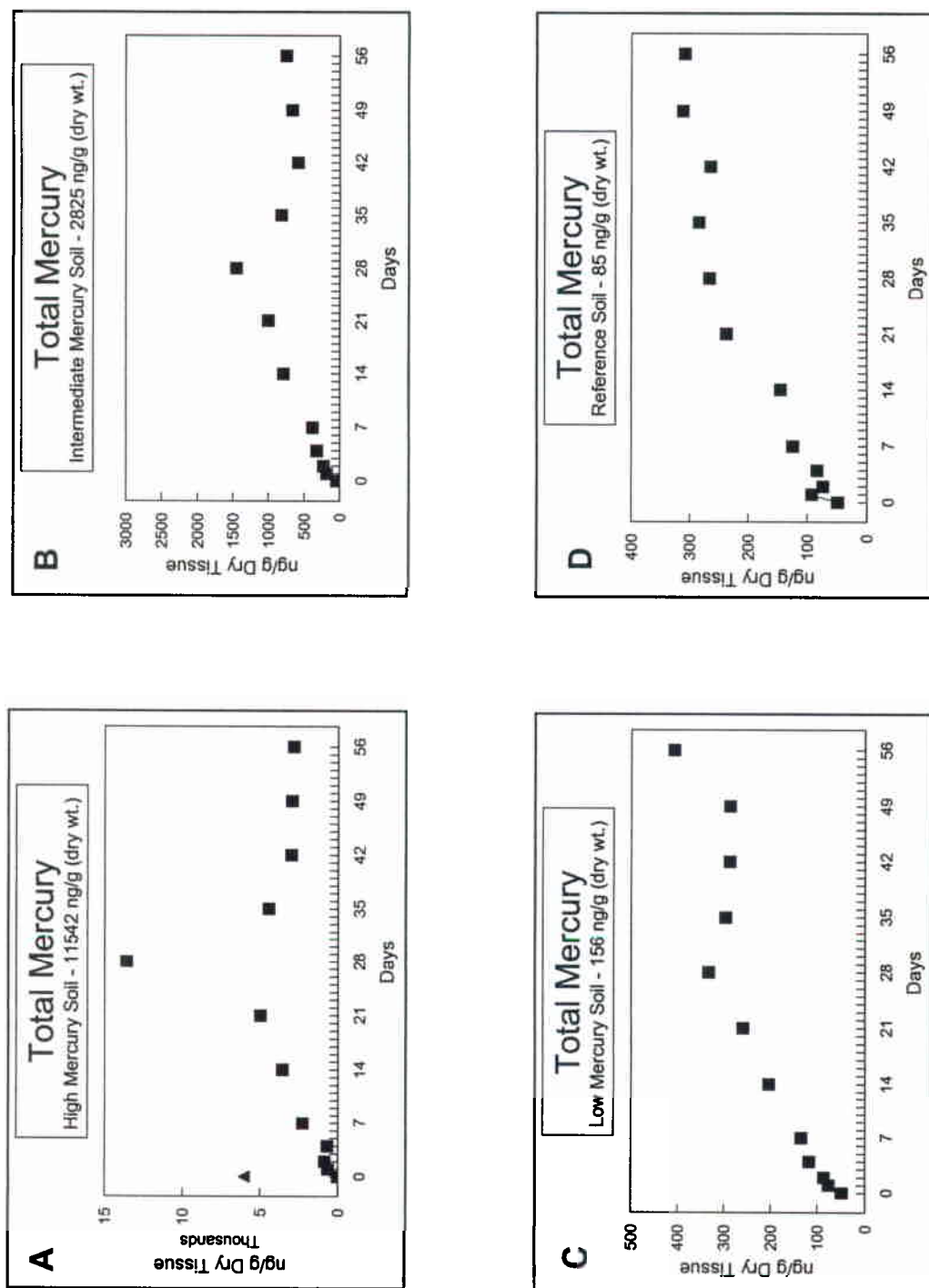


Figure 2-3. Total Mercury Uptake and Depuration in the Earthworm Exposed to the High (A), Intermediate (B), Low (C), and Reference (D) Soils. Legend: Squares- Mean of Four Replicates; Triangle- T-Hg in Earthworms Taken from Study Site.

A steady state did not occur during the 28-day uptake period. The BIOFAC model predicted the time to 90% steady state to be 38 and 41 days, respectively, in the high and intermediate treatments (Table 2-3). The 90% steady state for the earthworms held in the low and reference soil was predicted to be 30 and 36 days, respectively, when all data through day 56 were used as uptake data only. All data through day 56 were used as uptake data because the reference earthworms were exposed for 56 days to a constant concentration of T-Hg (85 ng/g dry weight soil) and reached a steady state. As shown below, no difference was found between the low and reference treatments in both the uptake and depuration phase; thus, the data through day 56 were used as uptake data.

Table 2-3. Total Mercury Bioaccumulation Factors and Associated Kinetic Data for Earthworms in the High, Intermediate, Low, and Reference Exposure Soils

Parameter	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
k_1	0.041	0.032	0.170	0.235
k_2	0.060	0.057	0.078	0.065
BAF	0.7	0.6	2.2	3.7
Time to 90% Steady State (days)	38	41	30	36
Time to 50% Clearance (days)	12	12	9	11
Heteroscedasticity	1.9690	0.8872	0.3583	0.1687

The shape of the uptake curve was similar for all four treatments (Fig. 2-3). The concentration at day 28 in the high T-Hg exposure (Fig. 2-3, Panel A) appears to be an outlier when one considers the arithmetic value; however, the value did not behave as an outlier in the logarithmic statistical model. A sensitivity analysis run on the day 28 data showed that when the T-Hg value at day 28 was reduced by 50%, no statistically significant difference occurred between the two data sets. The degree of curvature was not significantly different among treatments ($p < 0.6189$). This indicates that the kinetics controlling uptake were similar at all concentrations. Curvature was negative which shows that the rate of accumulation slowed over time for all treatments. The rate of T-Hg uptake, however, differed among treatments ($p < 0.0001$). All T-Hg treatments had uptake slopes that were positive and significantly greater than zero ($p < 0.0001$); thus, uptake increased

over time for all treatments during the 28-day uptake period (Appendix E, Table E-1). The slopes showed a monotonic increasing trend with increasing mercury concentration. A comparison of the high, intermediate, and low treatment slopes to the slope of the reference treatment showed that the rate of increase was significantly greater in the high ($p < 0.0001$) and intermediate ($p < 0.0035$) treatments relative to the reference (Appendix E, Table E-2). No difference occurred between the low and reference treatments ($p < 0.7895$).

The uptake of T-Hg over a 45-day exposure period has been studied by Helmke et al. (1979) in the earthworm *Aporrectodea tuberculata* using radioactive mercury ($^{203}\text{Hg}^{2+}$). The curvilinear uptake curve was similar to the uptake curves observed in the current study. Uptake appeared to be near steady state by day 45 which is similar to the steady state values observed in this study which ranged from 30 to 41 days (Table 2-3).

A comparison of the day 28 data between the tissue concentrations of the earthworms at each exposure treatment showed that the mean T-Hg concentrations in the earthworms exposed to both the high ($p < 0.0001$) and intermediate ($p < 0.0001$) soils were significantly higher than the reference mean (Appendix E, Table E-3). No difference ($p < 0.3106$) occurred between the low treatment and the reference mean. The quadratic term for the uptake phase had a coefficient of -0.00142 ($p < 0.0025$) which indicated that the logarithm of T-Hg concentration increased less rapidly over time.

The depuration of T-Hg in the high and intermediate treatments initially decreased from day 28 and reached a depuration steady state by day 35 (Fig. 2-3). No significant change occurred in T-Hg concentrations at the high ($p < 0.0531$) and intermediate ($p < 0.8400$) treatments from days 35 to 56. Total mercury in the low soil treatment appeared to initially decrease, reach a plateau and then start to increase by day 56 (Fig. 2-3); however, as shown below, no statistically significant change occurred during depuration. As was the case for the low treatment, T-Hg in the reference earthworms did not decrease during the 56-day exposure. The statistical analyses of the T-Hg depuration data showed a significant rate of decline of T-Hg in the high ($p < 0.0054$) and intermediate ($p < 0.0001$) treatments (Appendix E, Table E-4). Statistically, no difference occurred in the rate of change of the logarithm of T-Hg in the low ($p < 0.3588$) and reference ($p < 0.5723$) treatments.

The time to 50% clearance was predicted by the BIOFAC model to be 12 days for both the high and intermediate treatment (Table 2-3). BIOFAC estimated the time to 50% clearance would be 9 and 11 days, respectively, in the low and reference treatments. The time for 50% T-Hg clearance of 9-12 days for all treatments is reflected in the depuration rate constants (k_2) which ranged from 0.06-0.08 (Table 2-3). In contrast to the present study where time to 50% clearance was estimated to be 9 to 12 days, Helmke et al. (1979) estimated the biological half-life of radioactive ^{203}Hg to be 103 days. The half-life of 103 days is an order of magnitude longer than the time to 50% clearance estimates in the present study.

A comparison of the high, intermediate, and low T-Hg slopes to the reference treatment in the depuration phase (Appendix E, Table E-5) showed that a significant difference occurred in the rate of change between the high and reference treatments ($p < 0.0001$) and the intermediate and reference treatments ($p < 0.0104$). No difference occurred between the low and reference treatment ($p < 0.8212$). A comparison between the mean tissue concentrations of the earthworms at each exposure treatment on day 56 of the depuration phase showed that the high ($p < 0.0001$) and intermediate treatments ($p < 0.0001$) were significantly higher than the reference mean (Appendix E, Table E-6). The mean concentration of the low treatment was not significantly different from the reference ($p < 0.2644$) at day 56.

The depuration of T-Hg at the high and intermediate treatments followed a two phase elimination model. The chemical was eliminated rapidly from day 28 to day 35 followed by a depuration steady state. Neuhauser et al. (1995) found that copper, lead, and nickel are also eliminated rapidly between days 0 and 7 followed by a plateau for periods between 56 and 112 days in *A. tuberculata*. Likewise, Sheppard et al. (1997) found a biphasic initial "fast" phase followed by a "slow" plateau phase for iodine and manganese which lasted >80 days. In addition to iodine and manganese, Sheppard et al. (1997) also observed a fast and slow depuration phase for cadmium, cesium, and zinc. Cadmium, cesium, and zinc did not plateau during the slow phase. The investigators attributed the initial fast phase of depuration for all metals studied to gut clearance followed by slower physiologically mediated depuration from the tissues. The fast phase dominated the loss of metals from the earthworm during the first few days of depuration. The mean half-time for gut clearance was 1.4 days; the range was 0.94 to 2.7 days for the five metals. The mean half-time for physiological clearance of the tissues ranged from a low of 24 days for cesium and 40 days for manganese up to 150 days for cadmium. A gut clearance half-time for T-Hg could not be calculated in this study because there was a seven-day period between the time the earthworms were moved from the high and intermediate-contaminated soils to the reference soil and the first depuration measurements were made. Nevertheless, the fast phase for T-Hg in his study appears to be similar to other metals observed by Sheppard et al. (1997). No significant changes in T-Hg concentrations occurred from day 35 to day 56 in the high and intermediate treatments; thus, the physiologically mediated slow phase was >21 days for T-Hg in this study.

The BAFs (k_1/k_2) for the high and intermediate treatments were estimated to be 0.7, and 0.6, respectively, when the data through day 35 of depuration were used (Table 2-3). Bioaccumulation factors of 0.7 and 0.6 were estimated for the high and intermediate treatments by BIOFAC when all data through day 56 were used in the model. A BAF of 0.5 was obtained for the random sample of earthworms (species not identified) that were present at the time the high T-Hg soil sample was taken from the field for the study. The BAFs for the low and reference earthworms were estimated by BIOFAC to be 2.2 and 3.7, respectively, using all data through day 56 as uptake data. As discussed above, T-Hg uptake reached a steady state during the 56-day exposure period in both the low and

reference treatments. Likewise, there was no statistical difference between the two treatments from day 28 to day 56. Thus, the mean T-Hg tissue concentrations of the five sample periods for the low and reference treatments divided by the average soil concentrations were also used to estimate the BAFs. The mean tissue concentrations for days 28 to 56 were 323 and 291 ng/g dry weight tissue, respectively, for the low and reference earthworms. The mean concentrations of T-Hg in the low and reference soils were 156 and 85 ng/g dry weight soil. The BAFs calculated by using the mean tissue concentration divided by the mean concentration of T-Hg in the soil were 2.1 and 3.4, respectively, for the low and reference soil exposures.

The BAFs in this study were larger (2.1-3.7) for earthworms exposed to the soils containing low T-Hg concentrations of 0.085 mg/kg dry weight soil (reference soil) and 0.156 mg/kg dry weight soil (low T-Hg soil) than the BAFs (0.6-0.7) obtained in the intermediate and high T-Hg soils of 2.825 and 11.542 mg/kg dry weight soil. However, the absolute concentrations of T-Hg bioaccumulated by the earthworms were larger at the higher soil concentrations. Estimates of the T-Hg tissue concentrations at steady state, using the BAF shown in Table 2-3, were 8.079, 1.695, 0.343, and 0.315 mg/kg dry weight, respectively, at soil concentrations of 11.542, 2.825, 0.156, and 0.085 mg/kg soil (dry weight). A T-Hg concentration of 6.040 mg/kg dry weight soil was found in the random sample of earthworms taken from the field while obtaining the high mercury soil of 11.542 mg/kg dry weight for the study.

Larger BAFs in low T-Hg soils relative to low BAFs in soils containing higher concentrations of T-Hg can be seen in other studies where it is assumed the animals are at steady state. Sample et al. (1998 and 1999) developed a log-log regression model of T-Hg concentration in earthworms versus T-Hg concentration in the soil for earthworm data taken from four field studies (Bull et al. 1977; Helmke et al. 1979; Fisher and Koszorus, 1992; and Talmadge and Walton, 1993). The regression model showed that larger BAFs occurred at low soil concentrations. The model also showed that at ~1 mg/kg dry weight soil, all BAFs were <1 as the T-Hg concentrations in the soil increase.

The same trend can be seen in field data not used in the analysis by Sample et al. (1998 and 1999). Cocking et al. (1994) found higher concentrations of T-Hg in both *L. nubellus* and *L. terrestris* (guts voided 48 hours prior to analysis) as the concentration of mercury increased in the soil from ~0.5 to ~50 mg/kg dry weight soil. BAFs <1 were found in earthworms from 20 of 26 sample locations. Earthworms from four of 26 samples had BAFs <1.5; one sample had a BAF of ~3.5; and one sample had a BAF of ~5. In earthworms taken from soil with T-Hg concentrations which ranged from 0.1 to 0.5 mg/kg dry weight, the BAFs of 38 samples ranged (with the exception of two values <1) from 1 to 7.7. Total mercury BAFs calculated from field data in Ramos et al. (1999) for *Allolobophora molleri* (guts voided 48 hours) ranged from 2.7 to 13.1 for earthworms taken from soils which ranged from 0.08 to 0.9 mg/kg dry weight. One exception occurred in the Ramos et al. (1999) study, in which a BAF of 0.6 was calculated for earthworms exposed

0.11 mg/kg dry weight Cocking et al. (1991) found BAFs ranging from 0.6 to 1.6 in *Lumbricus* spp. (guts voided 24 hours) taken in the field from soils with T-Hg concentrations ranging from 8.2 to 22.0 mg/kg dry weight soil.

A number of factors have been postulated to explain why most heavy metals do not continue to bioaccumulate in earthworms taken from the field when soil concentrations are high. These factors include such things as age (growth) of earthworms at the time of exposure (Honda et al., 1984); avoidance behavior (Eijsackers (1987); differences in feeding habits and food selection of various species (Depta et al., 1999; Ireland, 1979); contaminant spatial variability (Marinussen et al., 1997b); toxicity (Svendsen and Weeks, 1997); changes in uptake as a function of seasonal exposure (Braunschweiler, 1995); and biological diversity among earthworms (Marinussen et al., 1997b). Two other factors which appear to control uptake and bioaccumulation of metals are metal bioavailability in soils and the physiological regulation of metals by the organism.

Metal bioavailability in soil has been shown to influence the rate of uptake of certain metals in earthworms. A number of physical, chemical, and biological factors can influence the bioavailability of various metals in soil (for ex., see Hesterberg, 1998; Peijnenburg et al., 1997; Rieuwerts et al., 1998; Sijm et al., 2000). The most important soil characteristics that have been shown to influence metal bioavailability to earthworms are pH, organic matter content, cation exchange capacity, and calcium concentration (Beyer et al., 1987; Ma, 1982; Ma et al., 1983; Peijnenburg et al., 1999a,b; Sample et al., 1998 and 1999; Sijm et al., 2000). Of the above factors, pH appears to be the most important and has been shown to modulate pore water-mediated uptake of cadmium in earthworms (Beyer et al., 1987; Ma, 1982; Ma et al., 1983; Peijnenburg et al., 1999a,b). Likewise, Ma (1982) and Ma et al. (1983) have shown that lowering pH can increase desorption of lead and zinc from the soil matrix and in turn increase the uptake and bioaccumulation of the metals. Janssen et al. (1997) have suggested that the BAFs for arsenic, cadmium, copper, and zinc in earthworms may be governed by the same soil characteristics that determine equilibrium partition coefficients between the solid phase and pore water.

In contrast to metal bioavailability which can influence the rate of metal uptake, physiological regulation by the organism has been shown to be a mechanism which can control bioaccumulation of metals in earthworms. Ireland (1979) originally proposed that copper, manganese, and zinc may be regulated in the tissues of *L. rubellus* because tissue levels did not reach concentrations present in contaminated soils. Copper has recently been shown to be regulated in *L. rubellus* (Svendsen and Weeks, 1997) and several other species (Marinussen et al., 1997a; Peijnenburg et al. (1999a,b). The regulation of nickel has been proposed for *E. fetida* (Fleckenstein and Graff, 1982) and *E. andrei* (Peijnenburg et al., 1999a). Other accumulation data also indicate that nickel may be regulated in several other species (Beyer et al., 1982; Gish and Christensen, 1973). Likewise, chromium appears to be regulated in several species of earthworms (Beyer and Chromartie, 1987; Helmke et al., 1979; Peijnenburg et al., 1999a; Pietz et al., 1984).

Cadmium is a notable exception to the physiological regulation of metals. Cadmium continues to bioaccumulate in tissues as soil concentrations increase in several species of earthworms (Honda et al. 1984; Peijnenburg et al., 1999b; Sheppard et al., 1997).

In the case of some metals, it is not clear how well the metals may be regulated. For example, it has been suggested that zinc is regulated in *E. andrei* and *E. crypticus* (Peijnenburg et al., 1999a,b). In contrast, it has been shown that zinc may not be regulated in *A. tuberculata* over a range of soil concentrations observed at contaminated sites (Neuhauser et al., 1995). Panda et al. (1999) have shown that zinc is bioconcentrated in *Drawida willsi* at soil concentrations <200 mg/kg dry weight, but the earthworms were able to regulate their tissue concentrations in soil containing 200-400 mg/kg dry weight.

The regulation of a metal at some threshold concentration in the soil like that observed by Panda et al. (1999) for zinc appears to occur for other metals as well. Marrinussen et al. (1997a) found that copper concentration increased proportionally in *D. veneta* with soil concentration up to ~150 mg/kg total extractable Cu. Copper was regulated in the earthworms exposed to higher concentrations in the soil. Total mercury appears to be regulated above a threshold of ~1mg/kg dry weight in the soil. The mechanism for such control is not clear. Neuhauser et al. (1995) have suggested that elimination rates for certain metals (e.g., copper, lead, and nickel) may increase as soil concentrations increase. The elimination rate constants (k_2) for T-Hg in this study ranged from 0.06 at the high T-Hg concentration of 11.542 mg/kg dry weight to 0.07-0.08 at the low and reference soil concentrations of 0.156 and 0.085 mg/kg dry weight, respectively (Table 2-3). These data suggest that elimination rates do not increase at higher soil concentrations and thus account for regulation at some threshold soil concentration.

Clearly, a better understanding of the mechanisms that control both uptake and elimination of metals is needed. It is imperative that the dynamics of the soil particulate/pore water phase of metals be better understood in order to predict the bioavailability and subsequent uptake of various metals. Total metal concentrations in the soil cannot be used in many cases to predict bioavailability, rates of uptake, or bioaccumulation. Several investigators have argued that certain metals, e.g., chromium, copper, manganese, nickel, and T-Hg are not as readily bioavailable as other metals which in turn would influence uptake and bioaccumulation (Helmke et al., 1979; Edwards et al., 1996; Peijnenburg et al., 1999a). The importance of the route of uptake, that is, earthworm digestive system versus skin, has not been systematically studied with regard to uptake and regulation of metals. Weltje (1998) states that the skin may be the dominant uptake route for metals. Fleming and Richards (1982) have shown that lead and iron can be adsorbed to the supracuticular mucoid coat and to a lesser extent the cuticle matrix of the skin of *E. fetida*. If this compartment is important, an understanding of the kinetics of skin adsorption and desorption will help clarify metal uptake and elimination kinetics in whole earthworms. The kinetics of internal depuration also needs to be better understood. Several studies

have shown that elimination rate kinetics are important in predicting steady state as well as the role of regulation of metals.

2.2.4 Bioaccumulation of Monomethylmercury

The uptake and depuration of MMHg by the earthworm in the high, intermediate, low, and reference soils are shown graphically in Figure 2-4, Panels A-D, respectively. The raw data for MMHg in the tissue of the earthworms exposed to the study soils are given in Appendix C. The uptake phase of MMHg followed first order reaction kinetics. As was the case for T-Hg, uptake did not reach a steady state in 28 days. The BIOFAC model predicted the time to 90% steady state to be 172, 192, and 97 days, respectively, in the high, intermediate, and low exposure soils (Table 2-4). The MMHg steady state estimates have a high degree of variability relative to the steady state estimates for T-Hg. No estimate of time to steady state was made for the earthworms in the reference soil because uptake was continuous and steady state did not occur during the 56-day exposure period.

The shape of the uptake curve was similar for all four treatments (Fig. 2-4). As was the case for T-Hg, the concentration at day 28 in the high MMHg exposure (Fig. 2-4, Panel A) appears to be an outlier; however, the logarithmic statistical model did not treat the point as an outlier. The degree of curvature was not significantly different among the treatments ($p < 0.7008$). This indicates that the factors governing uptake were the same at all concentrations. Negative curvature occurred for all treatments in the uptake phase which shows that the rate of uptake slowed over time. The rate of MMHg uptake, however, differed among treatments ($p < 0.0001$). All MMHg treatments had uptake slopes that were positive and significantly greater than zero ($p < 0.0001$) which shows that uptake increased over time for all treatments (Appendix F, Table F-1). The slopes also exhibited a monotonic increasing trend with increasing MMHg concentration. A comparison of the high, intermediate, and low treatment slopes to the reference slope showed that the rate of increase was significantly greater in the high ($p < 0.0184$) and intermediate ($p < 0.0412$) treatments relative to the reference (Appendix F, Table F-2). No difference occurred between the low and reference treatments ($p < 0.4674$).

A comparison on day 28 between the tissue concentrations at each exposure treatment showed that the mean MMHg concentrations in the earthworms exposed to the high ($p < 0.0001$), intermediate ($p < 0.0083$), and low ($p < 0.0360$) treatments were significantly higher than the reference mean (Appendix F, Table F-3). The quadratic term for the uptake phase had a coefficient of -0.00362 ($p < 0.0001$) which indicates that the logarithm of MMHg concentration increased less rapidly over time.

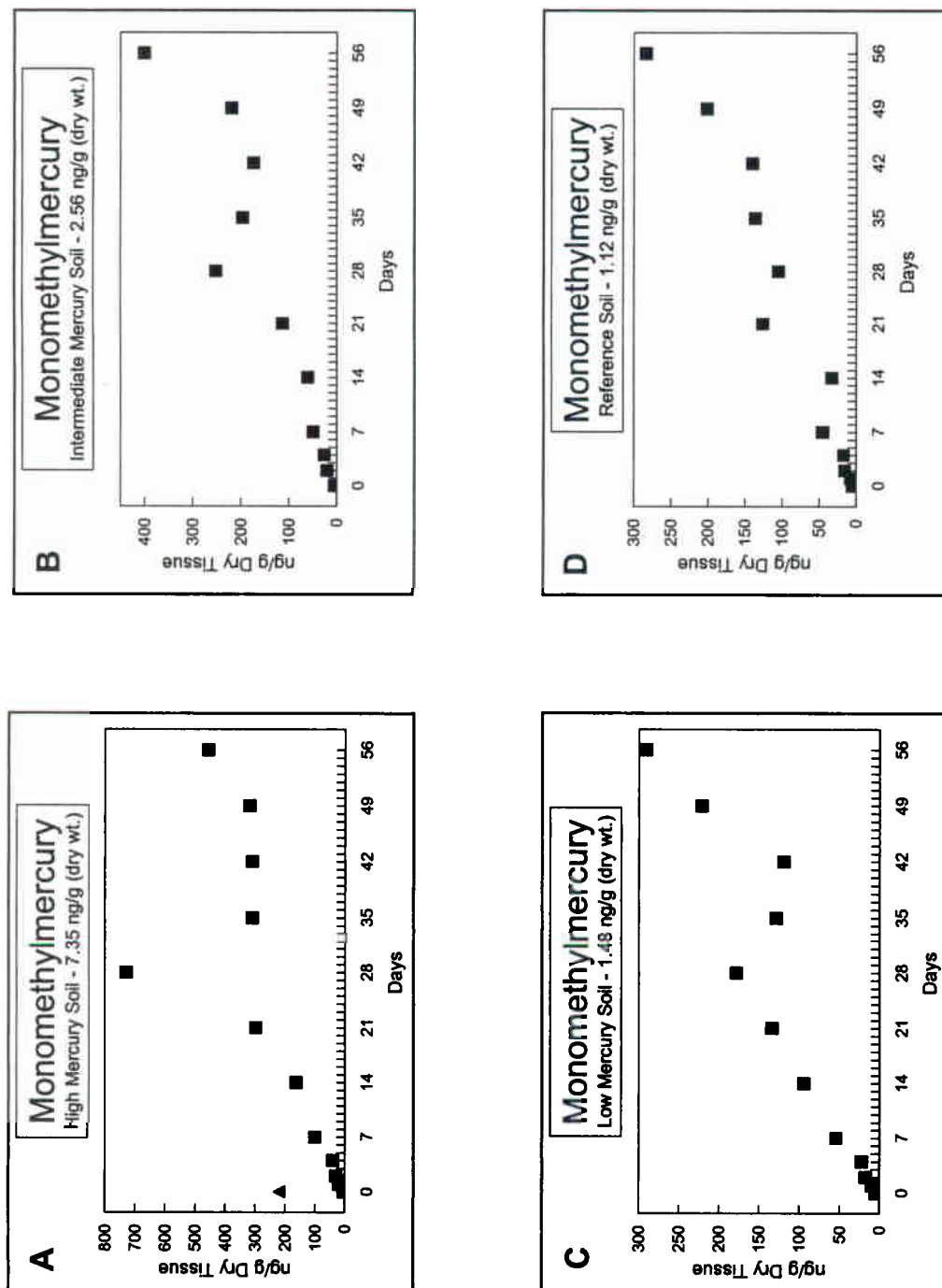


Figure 2-4. Monomethylmercury Uptake and Depuration in the Earthworm Exposed to the High (A), Intermediate (B), Low (C), and Reference (D) Soils. Legend: Squares- Mean of Four Replicates; Triangle- MMHg in Earthworms Taken from Study Site.

Table 2-4. Monomethylmercury Bioaccumulation Factors and Associated Kinetic Data for Earthworms in the High, Intermediate, Low, and Reference Exposure Soils

Parameter	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
k_1	2.331	2.757	5.565	^a
k_2	0.013	0.015	0.024	^a
BAF	179	184	232	260 ^b
Time to 90% Steady State (days)	172	192	97	^a
Time to 50% Clearance (days)	52	99	29	^a
Heteroscedasticity	2.1013	1.4116	1.8849	^a

^a Value not available.

^b Estimate made from the T-Hg uptake data through day 56 of depuration (see text).

The depuration of MMHg did not follow a two-phase model as was the case for T-Hg. In the high, intermediate, and low treatments, MMHg initially began to decrease but reversed direction and continued to increase (Fig. 2-4). The reference treatment increased throughout the depuration phase. Statistically, the rate of logarithmic change differed among treatments ($p < 0.0001$). The quadratic terms were positive and significant ($p < 0.0002$) for the high, intermediate, and low treatments which indicates that the rate of change in the depuration period increased after the initial drop in MMHg concentration. The quadratic term for the reference treatment was not significant ($p < 0.4877$) which implies that the rate of increase in the depuration period did not change, that is, the rate of increase (uptake continued throughout the depuration period) was constant. The linear terms for the intermediate, low, and reference treatments were positive which show that on average MMHg concentrations continued to increase during the depuration phase (Appendix F, Table F-4). In the high treatment, the linear term was negative which shows that on average a decrease occurred from day 28 to day 56.

A comparison of the high, intermediate, and low MMHg slopes to the reference treatment in the depuration phase (Appendix F, Table F-5) showed that the rate of increase was significantly less in the high ($p < 0.0001$) and intermediate ($p < 0.0108$) treatments

relative to the control treatment. No difference occurred between the low and reference treatment ($p < 0.0999$). A comparison of the mean tissue concentrations of the earthworms at each exposure treatment on day 56 of the depuration phase showed that the concentration of MMHg in the tissues of the high treatment earthworms was significantly higher ($p < 0.0059$) than in the reference earthworms (Appendix F, Table F-6). The mean concentrations of MMHg in the tissues of the intermediate ($p < 0.4409$) and low ($p < 0.0823$) treatment earthworms were not significantly different from the reference earthworms.

The time to 50% clearance was predicted by BIOFAC to be 52, 99, and 29 days, respectively, in the high, intermediate, and low treatments. The time to 50% clearance estimates are highly variable especially one considers that the depuration rates constants range from only 0.01-0.02 (Table 2-4). Spacie and Hamelink (1995) have pointed out that one drawback of nonlinear estimation procedures (e.g., BIOFAC) is that they do not provide an unique solution of the equation representing the model. Thus, there is always the chance that the iterative model will converge on a less rigorous answer, particularly if the initial parameter estimates are poor. This appears to be the case for both time to 90% steady state and time to 50% clearance.

The kinetic profiles for MMHg depuration at the high, intermediate, and low treatments were unexpected and complex in that the compound initially began to decrease but reversed direction and then continued to increase. We could find no other metal in the literature that has similar behavior during depuration with the possible exception of nickel. Neuhauser et al. (1995) transferred *A. tuberculata* from a contaminated soil to a clean soil to determine elimination rate constants for several metals. The concentration of nickel in the earthworm (gut voided 24 hours) at day 0 was 21.2 mg/kg dry weight. The concentration decreased to a low concentration of 3.2 mg/kg by day 20 then increased to 4.1 mg/kg at day 40, 4.5 mg/kg by day 58, and 7.7 mg/kg by day 112. Given that the data were not treated statistically; further speculation that nickel may behave as MMHg is not warranted.

The BAFs for the high, intermediate, and low MMHg treatments were 179, 184, and 232, respectively, using data through day 42 of depuration when net depuration was occurring (Table 2.4). A BAF of 30 was obtained for the random sample of earthworms (species not identified) that were present at the time the high mercury soil sample was taken from the field for the study. The BAF for the reference earthworms was estimated as follows. Monomethylmercury bioaccumulation continued to increase throughout the 56-day exposure period. As shown above, a steady state occurred in T-Hg bioaccumulation in the reference earthworms. Thus, the worst case assumption was made that the MMHg concentration in the reference earthworms eventually comprised 100% of the T-Hg concentration in the reference earthworms at steady state. The mean T-Hg tissue concentration at steady state was 291 ng/g dry weight tissue. The assumption was made that the MMHg concentration was also 291 ng/g dry weight tissue; thus, the BAF would be 260 (291 ng/g dry weight tissue divided by 1.12 ng/g dry weight soil).

Beyer et al. (1985) demonstrated that MMHg can bioaccumulate in *E. fetida*. Earthworms were exposed for 12 weeks to soils containing 0.63 (control) and 1.3 mg/kg mercury wet weight and 3.8 mg/kg mercury for 6-12 weeks. The mercury measurements were made as T-Hg via cold vapor atomic absorption spectrophotometry. Assuming that no MMHg was present in the "Baccto" potting soil used for all treatments, we subtracted 0.63 mg/kg T-Hg measured in the control soil from the mercury in the treatment soils to correct to MMHg. We have also assumed that the remaining mercury is all MMHg. Likewise, we corrected the mercury concentration in the MMHg exposure earthworms by subtracting the T-Hg concentration found in the control earthworms. We took the liberty of calculating BAFs from the data since the exposure time of 84 days in the Beyer et al. (1985) study was close to the lowest steady state time of 97 days predicted in this study. The BAFs of the earthworms exposed to the MMHg-corrected soils of 0.67 and 3.17 mg/kg (wet weight) soils (when earthworms and soils were corrected to dry weight) were 84 and 91, respectively. The BAFs were lower than those predicted from the current study. Based on the steady state data from the current study, the earthworms in the Beyer et al. (1985) study were not at steady state. Thus, the BAFs would be expected to be lower than those obtained at steady state.

The MMHg BAF of the earthworms taken from the field when the soil was initially procured for the study was 30 (220 ng/g in the earthworms divided by 7.35 ng/g in the soil) which is considerably less than the range of 179-260 predicted by the model. If one assumes the earthworms taken from the field were at steady state, it appears that the model is overestimating the bioaccumulation of MMHg. The assumption is being made that the earthworms were at equilibrium since the T-Hg BAF of 0.5 fell within the range predicted by the model for the concentration of T-Hg in the soil. The BAFs from the field earthworms, those from Beyer et al. (1985) and the current study are all within an order of magnitude of each other.

The concentrations of MMHg in the earthworms at steady state estimated from the BAFs are approximately 1.316, 0.471, 0.343, and 0.291 mg/kg dry weight tissue, respectively, in the high, intermediate, low, and reference earthworms. The percentage of MMHg relative to T-Hg based on T-Hg tissue concentrations of 8.079, 1.695, 0.343, and 0.315 mg/kg dry weight tissue in the high, intermediate, low, and reference earthworms is 16.3, 27.8, 100, and 92.4%, respectively. The percent of MMHg relative to T-Hg in the random sample of earthworms (species not identified) that were present at the time the high mercury soil sample was taken from the field for the study is 3.6%. Bull et al. (1977) found that the concentration of MMHg in *L. terrestris* taken from a mercury-contaminated study area in the field comprised 8-13% of the T-Hg in the earthworms. Based on the lower percentages MMHg relative to T-Hg obtained for the two field earthworm populations which are assumed to be at steady state, it appears that the model-derived BAFs may be highly conservative.

The absolute concentrations of MMHg in the earthworms were lower in the lower mercury-contaminated soils; however, the percentage of MMHg relative to T-Hg was higher as the concentration of T-Hg decreased. The percentage of MMHg in the soils relative to T-Hg were 0.06, 0.09, 0.95, and 1.32% in the high, intermediate, low, and reference soil respectively. The average percentage of methylmercury to T-Hg in soils generally ranges between 0.5 and 1.5% (Boudou and Ribeyre, 1997). Schwesig et al. (1999) have reported that MMHg comprised ~0.1 % of T-Hg in upland forest floor litter and 0.2-0.4% of the T-Hg in mineral soil which are similar to the lower values found in the current study.

The concentrations of MMHg found in this study from the same soil type and soil horizon may be a function of soil pH. The pH of the soils when taken from field, before adjustment to ~6 to run the exposure assays (Sect. 2.2.1), were 7.5, 7.1, 5.9, and 4.4 for the high, intermediate, low, and reference soils, respectively (Table 2-1). Yin et al. (1997) have shown MMHg adsorption as a function of pH follows a concave downward profile with the maximum adsorption to soils occurring between pH 5 and 6. Although this is speculation on our part, this may account for the higher concentrations observed in the lower pH soils. Organic matter and clay minerals also influence the adsorption of MMHg (Desauziers et al., 1997; Hempel et al., 1995; Hogg et al., 1978); however, total organic carbon and cation exchange capacity varied among the four soils (Table 2-1).

The uptake of MMHg followed predictable first order rate kinetics for both metals and most organics (Jager, 1998). The behavior of MMHg during depuration and its subsequent influence on bioaccumulation, however, is difficult to explain. Relative to several metals discussed above for T-Hg, MMHg is not well regulated by the earthworm. One may speculate that regulation of MMHg may be different from most metals because it is an organic which may partition to fat. Based on the octanol-water partition coefficient for MMHg ($\log K_{ow}$ ranges from ~1.6 at pH 4 down to ~0.4 at pH 8; Major and Rosenblatt, 1991), some bioaccumulation should occur in the fat compartment. The arguments made above for T-Hg for a better understanding of the mechanisms that control both bioavailability in the soil and the mechanisms that control both uptake and elimination are also relevant for MMHg. In particular, a more comprehensive understanding of the kinetics of depuration is needed in order to obtain better BAFs. Additional MMHg data taken from earthworms in the field would help to clarify MMHg concentrations at steady state. Well established BAFs for MMHg are needed to more adequately assess ecological impact at mercury-contaminated field sites.

3. ECOLOGICAL RISK OF MERCURY TO THE ROBIN AND SHREW

Mercury-contaminated soil (0-15 cm horizon; 0-6") in the vicinity of the Northeast Test Hut has total mercury concentrations which range from ~0.1 mg/kg dry weight (background) up to ~15 mg/kg dry weight (Dames & Moore, Inc., 1997a,b). A baseline ecological risk assessment (ERA) of the mercury contamination concluded that avian and mammalian species could be at risk when exposed to mercury via the ingestion of terrestrial life that accumulates the contaminant (Dames & Moore, Inc., 1998). As discussed in Section 1 of this report, EPA Region III established a total mercury clean up level of 0.1 mg/kg dry weight soil for the Northeast Test Hut because of the contamination at the site. EPA Region III also gave the Army the option of conducting bioaccumulation studies with earthworms exposed to contaminated soil at the Northeast Test Hut to determine the bioaccumulation factors for mercury which could be used to more accurately assess the risk identified in the ERA to wildlife which may feed on earthworms at the site. Because of the lack of appropriate data, the ERA made the conservative assumption that all mercury at Graces Quarters was in the methylated form (MMHg) which is the most toxic form of mercury found in soil. The objective of Section 3 was to re-evaluate the conclusions reached in the ERA using the MMHg BAFs established in Section 2. The same receptors, exposures, ecological effects, risk characterization, uncertainties, etc., used in the original Graces Quarter ERA (Dames & Moore, Inc., 1998), have been used in the re-assessment.

3.1 Problem Formulation

The site description, identification of mercury as a potential problem, identification of the exposure pathways, and identification of assessment and measurement endpoints are covered in detail in the ERA problem formulation phase (Dames & Moore, Inc., 1998); thus, they will not be repeated in this report. Briefly, the following ecological receptors were selected for study in the ERA. Earthworms were selected to represent terrestrial invertebrates because they have intimate contact with and ingest large amounts of surface soil; thus, they have the greatest potential to accumulate mercury from the soil relative to other terrestrial invertebrates (Dames & Moore, Inc., 1998). The American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*) were selected for evaluating potential effects to avian and mammalian receptor species because 1) a large portion of their diet is comprised of soil invertebrates relative to other birds and small mammalian species at Graces Quarters; 2) both species are present at Graces Quarters throughout the year; and 3) both species have limited foraging ranges, increasing their potential exposure to mercury at Graces Quarters (Dames & Moore, Inc., 1998).

3.2 Exposure Assessment

The purpose of the exposure assessment is to identify the concentration and/or dose of mercury to which the ecological receptors selected in the ERA could be exposed. The methods used below to calculate the maximum dose of mercury that a robin or shrew could be expected to obtain from the ingestion of earthworms at Graces Quarters follows that which is given in the original ERA (Dames & Moore, Inc., 1998). The following methods and equations, which were based on EPA risk assessment guidance for Superfund (U.S. EPA, 1989; as cited in Dames & Moore, Inc., 1998), were taken from the ERA (Dames & Moore, Inc., 1998):

The following equation was used to calculate the maximum dose of MMHg that a robin or shrew would be expected to obtain from the ingestion of contaminated earthworms:

$$\text{Dose}_{\text{worm}} = \text{FI} * \text{C}_{\text{diet}}$$

where

$\text{Dose}_{\text{worm}}$ = amount of MMHg ingested per day via the ingestion of earthworms (mg/kg bw-d);
FI = food ingestion rate (kg/kg bw-d); and
 C_{diet} = estimated maximum MMHg concentration in diet (mg/kg wet weight).

A food ingestion rate (FI) for robins was determined by using an allometric equation reported by Nagy (1987; as cited in Dames & Moore, Inc., 1998) for passerine birds:

$$\text{FI} = 0.398W^{0.850}$$

where

W = wet weight of robin (g).

Using a mean body weight of 77.3 g, as reported by Clench and Leberman (1978; as cited in Dames & Moore, Inc., 1998) for robins in Pennsylvania, the FI would be 16.03 g per day. The FI was converted to 0.21 kg/kg bw-d (16.03g/77.3g) in the ERA by using the body weight reported by Clench and Leberman (1978; as cited in Dames & Moore, Inc., 1998). An FI of 0.62 kg/kg bw-d, which was taken from Morrison et al. (1957; as cited in Dames & Moore, Inc., 1998), was used in the ERA for adult shrews. According the ERA, the above food ingestion rates were selected for use in the ERA because the rates are the highest ingestion rates found in the scientific literature; thus, they are likely to represent conservative estimates of exposure.

The estimated dietary concentration (C_{diet}) was calculated using the following equation:

$$C_{\text{diet}} = P_e * C_e$$

where

P_e = proportion of diet consisting of earthworms (unitless) and
 C_e = estimated concentration of MMHg in earthworms (mg/kg wet weight).

Two scenarios were used in the ERA for the proportion of the diet (P_e) consisting of earthworms (Dames & Moore, Inc., 1998). The first scenario assumed that 100% of the robin's and shrew's total diet was comprised of earthworms. As pointed out in the ERA, this assumption is conservative and may lead to an overestimate of potential risks because robins and shrews will likely obtain some food from areas other than those at the Northeast Test Hut. The proportion of diet consisting of earthworms was also evaluated in a less conservative, but more realistic second scenario, using dietary information from the scientific literature. The less conservative estimates, the derivations of which are described in detail in the ERA, were 22% for the robin and 31.4% for the shrew.

The estimated maximum concentration of MMHg in an earthworm (C_e) as fresh weight (wet weight) was determined using the following equation:

$$C_e = C_{\text{soil}} * \text{BAF}$$

where

C_{soil} = maximum concentration of MMHg detected in surface soil (mg/kg dry weight) and
 BAF = bioaccumulation factor for MMHg in earthworms (unitless).

The concentrations of MMHg found in the high, intermediate, and low mercury soils at the Northeast Test Hut were 0.00735, 0.00256, and 0.00148 mg/kg dry weight, respectively (Sect. 2.2.1). The concentration of MMHg in the Graces Quarter reference soil was 0.00112 mg/kg dry weight. The BAFs for earthworms exposed to MMHg in the high, intermediate, low, and reference soils were 179, 184, 232, and 260, respectively (Sect. 2.2.4). To be conservative, the estimated concentration of MMHg in earthworms found in the high, intermediate, low, and reference soils was calculated by using the highest BAF (260) obtained in the bioaccumulation study (Sect. 2.2.4). The estimated maximum concentrations of MMHg in earthworms (C_e) at the Northeast Test Hut in each contaminated soil are shown in Tables 3-1 and 3.2 for the robin and shrew, respectively. The C_e is normally expressed as mg/kg bw-d wet weight (Dames & Moore, Inc., 1998). Thus, the C_e data which were initially calculated for dry weight were corrected to wet

weight by multiplying the results of the above equation by a factor of 0.1, which was based on a report by Gibbs et al. (1996) which showed that 90% of *E. fetida*'s fresh weight is water.

Table 3-1. Baseline Risk Assessment Calculations for the American Robin Exposed to Four Contaminated Soils^a

Parameter	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
22% Earthworm Diet				
C_e	0.19110	0.06656	0.03848	0.02912
C_{diet}	0.04204	0.01464	0.00847	0.00641
$\text{Dose}_{\text{worm}}$	0.00883	0.00307	0.00178	0.00135
$\text{Dose}_{\text{soil}}$	0.00011	0.00004	0.00002	0.00002
$\text{Dose}_{\text{total}}$	0.00894	0.00311	0.00180	0.00137
100% Earthworm Diet				
C_e	0.19110	0.06656	0.03848	0.02912
C_{diet}	0.19110	0.06656	0.03848	0.02912
$\text{Dose}_{\text{worm}}$	0.04013	0.01398	0.00808	0.00612
$\text{Dose}_{\text{soil}}$	0.00011	0.00004	0.00002	0.00002
$\text{Dose}_{\text{total}}$	0.04024	0.01402	0.00810	0.00614

^a Units in mg/kg bw-d wet weight

Table 3-2. Baseline Risk Assessment Calculations for the Short-Tailed Shrew Exposed to Four Contaminated Soils^a

Parameter	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
31.4% Earthworm Diet				
C _e	0.19110	0.06656	0.03848	0.02912
C _{diet}	0.06001	0.02090	0.01208	0.00914
Dose _{worm}	0.03721	0.01296	0.00749	0.00567
Dose _{soil}	0.00043	0.00015	0.00009	0.00007
Dose _{total}	0.03764	0.01311	0.00758	0.00574
100% Earthworm Diet				
C _e	0.19110	0.06656	0.03848	0.02912
C _{diet}	0.19110	0.06656	0.03848	0.02912
Dose _{worm}	0.11848	0.04127	0.02386	0.01805
Dose _{soil}	0.00043	0.00015	0.00009	0.00007
Dose _{total}	0.11891	0.04142	0.02395	0.01812

^a Units in mg/kg bw-d wet weight

In addition to the ingestion of MMHg accumulated in earthworms, robins and shrews may also be exposed to MMHg through the inadvertent ingestion of surface soils while forging or grooming (Dames & Moore, Inc., 1998). The following equation was used in the ERA to calculate the dose of MMHg that the receptors could obtain from the ingestion of soil:

$$\text{Dose}_{\text{soil}} = \text{SI} * \text{C}_{\text{soil}}$$

where

Dose_{soil} = amount of MMHg ingested per day from soil (mg/kg bw-d);
 SI = soil ingestion rate (kg/kg bw-d); and
 C_{soil} = maximum MMHg concentration in surface soil (mg/kg).

As discussed in the ERA, it was assumed that 7.3 and 9.4% of the robin's and shrew's total mass of diet is soil (Beyer et al., 1994; as cited in Dames and Moore, Inc., 1998). The percent soil ingestion was multiplied by the food ingestion rates (FI) presented above to estimate soil ingestion rates. The estimated soil ingestion rate for the robin is 0.015 mg/kg bw-d (0.21 mg/kg bw-d (FI) * 0.073 mg/kg bw-d (percent of total mass of diet which is soil)). The estimated soil ingestion rate for the shrew is 0.058 mg/kg bw-d (0.62 mg/kg bw-d (FI) * 0.094 mg/kg bw-d (percent of total mass of diet which is soil)). The estimated $Dose_{soil}$ for the robin and shrew is given in Tables 3-1 and 3-2, respectively, for the four soils evaluated at the Northeast Test Hut.

The total dietary exposure levels for the robin and shrew to MMHg was determined by the following equation (Dames & Moore, Inc., 1998):

$$Dose_{total} = Dose_{worm} + Dose_{soil}$$

The estimated total doses of MMHg using the above equation from the ingestion of MMHg in earthworms and surface soil for the robin and shrew in each treatment soil at the Northeast Test Hut are given in Tables 3-1 and 3-2, respectively. The estimated $Dose_{total}$ for each receptor was calculated for diets which assume 1) the robin's and shrew's diet are composed of 22 and 31.4% earthworms, respectively, and 2) both the robin's and shrew's diets consist of 100% earthworms. The total dietary intakes of each receptor has been used in a comparison of dietary toxicity values in the risk characterization section (Sect. 3.4) following the same analysis given in the ERA (Dames & Moore, Inc., 1998)

3.3 Ecological Effects Assessment

Toxicity criteria for MMHg have not been developed by EPA for terrestrial species (Dames & Moore, Inc., 1998). Thus, toxicity data in the scientific literature were reviewed during the ERA process to characterize the toxicity of MMHg. As discussed in the ERA, toxicity values selected for the evaluation of the potential for adverse effects are referred to as toxicity reference values (TRVs) and represent concentrations of chemicals of potential concern that are assumed to be protective of the ecological receptors being evaluated. Toxicological benchmarks derived by Opresko et al. (1994; as cited in Dames & Moore, Inc., 1998) were used in the ERA to evaluate the potential for adverse effects to the robin and shrew. According to the ERA, Opresko et al. (1994; as cited in Dames & Moore, Inc., 1998) reported a TRV for methylmercury of 0.012 mg/kg bw-d for the robin and a TRV of 0.151 mg/kg bw-d for the shrew. As discussed in Section 1 of this report, MMHg is the dominant species of methylmercury in soil. The above TRVs have been used to evaluate the potential for adverse effects to robins and shrews from the ingestion of earthworms at the Northeast Test Hut.

3.4 Risk Characterization

In the risk characterization process the potential exposure concentrations derived in the exposure assessment phase (Sect. 3.2) are compared with the TRVs derived in the ecological effects phase (Sect. 3.3) to evaluate the potential for adverse effects to receptors of concern. Estimated exposure concentrations for MMHg are compared to TRVs by creating a ratio of the estimated exposure concentrations to the TRV:

$$EEQ = \text{Dose}_{\text{total}} / \text{TRV}$$

where

EEQ	=	environmental effects quotient;
Dose _{total}	=	estimated exposure concentrations for MMHg; and
TRV	=	toxicity reference value.

If the EEQ is equal to or greater than 1.0, there is a potential for adverse effects to occur. The confidence of the conclusion increases as the magnitude of the ratio departs from 1.0 (Dames & Moore, Inc., 1998). The EEQs for the robin and shrew are summarized in Table 3-3.

The EEQs for robins from the ingestion of MMHg in earthworms and all surface soils at the Northeast Test Hut are <1 when earthworms were assumed to comprise 22% of a robin's diet. The EEQs for the robin were >1 from the ingestion of MMHg in earthworms and soil at the high- and intermediate-contaminated sites when it was assumed that earthworms comprised 100% of the robin's diet. The EEQs were <1 from the ingestion of MMHg in earthworms and soil at the low-contaminated and reference sites when it was assumed that earthworms comprised 100% of the robin's diet. The less conservative, but more realistic scenario of 22% earthworms in the robin's diet indicates that MMHg at the Northeast Test Hut will not adversely affect robins that ingest of MMHg in earthworms.

The conservative scenario of 100% earthworms in the robins's diet indicates that robins would be affected by MMHg at the Northeast Test Hut if they fed exclusively on earthworms from soils containing MMHg at concentrations of ~0.00256 mg/kg dry weight or higher. This assumption is highly conservative because the robin is likely to obtain earthworms over a large area of Graces Quarters. In fact, robins may obtain earthworms from areas other than those on Graces Quarters (Dames & Moore, Inc., 1998). The areal extent of existing mercury contamination at the Northeast Test Hut is quite small relative to the total area of the Northeast Test Hut. It is orders of magnitude smaller when one includes the entire land mass of Graces Quarters. As an example, the highest concentration of mercury (~11.5 mg/kg dry soil) in the 0-15 cm horizon is <1 m² at the Northeast Test Hut (Dames & Moore, Inc., 1997b).

The EEQs for shrews from the ingestion of MMHg in earthworms and all surface soils at the Northeast Test Hut were <1 when earthworms were assumed to comprise 100% of a shrew's entire diet and the less conservative 31.4% earthworm diet. Based on these results, it is reasonable to conclude that shrews will not be adversely affected by the ingestion of MMHg in earthworms and surface soils at the Northeast Test Hut.

Table 3-3. Environmental Effects Quotients (EEQs) for the American Robin and Short-Tailed Shrew Exposed to Four Contaminated Soils

Diet	High Mercury Soil	Intermediate Mercury Soil	Low Mercury Soil	Reference Soil
Robin				
22% Earthworms	0.75	0.26	0.15	0.11
100% Earthworms	3.35	1.17	0.68	0.51
Shrew				
31.4% Earthworms	0.25	0.09	0.05	0.04
100% Earthworms	0.79	0.27	0.16	0.12

3.5 Uncertainties

A number of the uncertainties associated with the estimates of ecological risks to the robin and shrew from consuming earthworms and soil contaminated with mercury have been discussed in the ERA (Dames & Moore, Inc., 1998). As in the case of the original ERA, the general approach in this evaluation has been to err on the side of conservatism. Thus, the risks are likely to be overestimated rather than underestimated. The following are areas of uncertainty which should be expressed to place the estimated risks into proper perspective.

The ERA made the point that a number of uncertainties are associated with the potential receptors species at Graces Quarters (Dames & Moore, Inc., 1998). We have made the assumption that the robin and shrew are appropriate representative avian and mammalian species as argued in the ERA. An area of uncertainty that may be important is the life stage of the receptors. The daily dose levels determined above are normalized to the body weight of the test animals. The normalization of toxicity data on a mg/kg bw-d

basis allows comparisons across tests and across species with appropriate consideration for differences in body size (Sample et al., 1996). It is well known, however, that several physiological functions, such as metabolic rate, are a function of body size. Early life stages generally have higher metabolic rates but are more resistant to toxic chemical because of more rapid rates of detoxification. However, as pointed out by Sample et al. (1996), this may not be case if the toxic effects of the chemical are produced primarily by a metabolite(s). If MMHg is detoxified before elimination and the resultant metabolite(s) is more toxic than the parent compound, the risk to early life stages may be underestimated if one assumes that the diet is composed entirely of contaminated earthworms. On the other hand, the exposure assessment assumed that seasonal ingestion rates were constant which over a yearly cycle is conservative and likely to overestimate the potential for adverse effects.

The ERA presented a good argument for selecting the earthworm as an important representative terrestrial invertebrate that will be in intimate contact with the mercury in the contaminated soils at the Northeast Test Hut. The question raised in the ERA as to whether or not earthworms are able to survive and reproduce at the higher mercury concentrations detected in the soils at the Northeast Test Huts can now be qualified. As discussed in Section 2.1.1 of this report, earthworms were obtained from soil which contained the highest concentration of total mercury at the site (~11.5 mg/kg dry weight). All life stages of earthworms, including cocoons, juveniles, and sexually mature adults were found in the highest mercury contaminated soil. Likewise, the data obtained in the bioaccumulation phase of this study showed that the test earthworms were not affected (as measured by growth) by exposure to the highest concentration of mercury (Sect. 2.2.2).

As discussed in the ERA, there is uncertainty associated with the potential exposure pathways selected for evaluation. The potential adverse effects to terrestrial wildlife from dermal absorption or inhalation of mercury exists; however, those pathways could not be evaluated because of the lack of exposure data. The ERA argues that these potential exposure pathways are unlikely to occur or result in adverse effects to the robin and shrew. Thus, inclusion of these pathways is unlikely to alter the risk estimations (Dames & Moore, Inc., 1998).

There is uncertainty associated with concentrations of mercury that the robin and shrew will be exposed to from the ingestion of earthworms and contaminated soil. As discussed above (Sect. 3.4), the areal extent of existing mercury contamination at the Northeast Test Hut is quite small relative to the total area of the Northeast Test Hut. It is orders of magnitude smaller when one includes the entire land mass of Graces Quarters. The uncertainty increases when one considers that the robin may feed part of the time at sites not located on Graces Quarters. Based on the foraging patterns of the robin and shrew, it is highly unlikely that the animals will consume 100% of their diets from earthworms which inhabit the small areas of mercury-contaminated soil. The surface soil at the most heavily contaminated site in the Northeast Test Hut area (i.e., the high mercury

sample) was essentially removed in order to conduct the bioaccumulation studies. Assuming that no other soil samples have mercury concentrations as high as the sample removed, the uncertainty is reduced that the receptors will be exposed to soil and earthworms at a similar concentration which poses a risk.

It was conservatively assumed that the MMHg BAF was 260 for all earthworms and that the BAF would be the same for those species found at the Northeast Test Hut. The BAFs for earthworms exposed to MMHg in the high, intermediate, low, and reference soils were 179, 184, 232, and 260, respectively (Sect. 2.2.4). To be conservative, the estimated concentration of MMHg in earthworms found in the high, intermediate, low, and reference soils was calculated by using the highest BAF (260) obtained in the bioaccumulation study (Sect. 2.2.4). If one uses the actual BAFs for each soil concentration, the estimated concentration of MMHg in the earthworms would be lower for those earthworms in the high, intermediate, and low exposure soils. Thus, the EEQs estimates would be lower for both the robin and shrew in all cases except the reference soil. As an example, the EEQ for robins consuming 100% earthworms in the intermediate soil would be <1 (EEQ = 0.83). However, as discussed in Section 2.2.4, there is statistical uncertainty associated with the BAFs. Thus, the more conservative BAF of 260 was used for all analyses.

As pointed out in the ERA, there are uncertainties associated with the assessment of risks when extrapolating from individuals to population and community levels. The following discussion, which summarizes the uncertainties, was taken directly from the ERA (Dames & Moore, Inc., 1998):

The most apparent uncertainty is the extrapolation of assumptions about the potential for adverse effects from individual organisms to populations or communities. For the higher trophic level terrestrial species, the ERA made conclusions about the potential for adverse effects to individual organisms. Very few models are available to extrapolate the potential for adverse effects from the individual level to the population or community-level. Because of the limited availability of such models, certain assumptions had to be made about the overall potential for adverse effects to ecological receptors. It was generally assumed if there is no potential for direct adverse effects to individual organism there is unlikely to be the potential for direct adverse effects to populations or communities. Similarly, it was assumed that if there is the potential for adverse effects to individual organisms there is also the potential for adverse effects to populations or communities. Risks may have been overestimated by this latter assumption.

3.6 Summary of the Environmental Assessment

The following assessment endpoints were used in the present assessment of mercury at the Northeast Test Hut:

- Adverse effects to terrestrial invertebrate communities (as represented by earthworms) from direct contact with MMHg in surface soil.
- Adverse effects to carnivorous birds (as represented by robins) from the ingestion of MMHg that had accumulated in terrestrial invertebrates (as represented by earthworms) and from the direct ingestion of MMHg in surface soil.
- Adverse effects to small mammals (as represented by shrews) from the ingestion of MMHg that had accumulated in terrestrial invertebrates (as represented by earthworms) and from the direct ingestion of MMHg in surface soil.

Soil-dwelling invertebrates may be exposed to mercury in surface soils at the Northeast Test Hut. The bioaccumulation of T-Hg and MMHg in earthworms was determined in soils taken from the Northeast Test Hut site. The study soils included soil with the highest concentration of mercury found at the Northeast Test Hut, an intermediate, and low concentration of mercury. Bioaccumulation factors were determined for both T-Hg and MMHg in all soils. Bioaccumulation factors for MMHg were used in this assessment since it is the most toxic species of mercury found in the soils at the Northeast Test Hut. The question of mercury toxicity to earthworms was raised in the ERA (Dames & Moore, Inc., 1998). During the determination of the BAFs, the study showed that mercury from the surface soil with the highest concentration found at the Northeast Test Hut was not toxic to the test earthworms. All life stages of earthworms, including cocoons, juveniles, and sexually mature adults were found for endemic earthworms in the highest mercury contaminated soil. These data indicate that soil invertebrates are unlikely to be adversely affected by the presence of mercury at the Northeast Test Hut.

The potential risk to robins and shrews are as follows. No risk was found for robins which have an annual diet that is comprised of 22% contaminated earthworms and soil from any site at the Northeast Test Hut. The highly conservative analysis that assumed a robin's annual diet consisted of 100% contaminated earthworms and soil from the highest and intermediate mercury-contaminated sites at the Northeast Test Hut showed that robins would be at risk. The risk for exposure to contaminated worms and soil at the highest mercury concentration detected at the Northeast Test Hut has been eliminated. The soil was removed from the site for use in the bioaccumulation study. Several small surface areas (<1 m²) exist at the site which contain concentrations of mercury similar to the intermediate concentration evaluated in the risk assessment. As discussed above, the

areal extent of existing mercury contamination at the Northeast Test Hut is quite small relative to the total area of the Northeast Test Hut. It is orders of magnitude smaller when one includes the entire land mass of Graces Quarters. The uncertainty decreases when one considers that the robin may feed at other uncontaminated sites on Graces Quarters. The current risk analysis shows that it is reasonable to conclude that shrews will not be adversely affected by the ingestion of MMHg in earthworms and surface soils found at the Northeast Test Hut.

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Appendix A

CHEMICAL ANALYSES OF GRACES QUARTERS' STUDY SOILS

Table A-1. General Chemical Characteristics of the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On-Site Reference
Ammonia (as N)	350.1	2.5	15.8	7.6	5.9	6.4
Cation Exchange Capacity	9081/6010	No reporting limits	17.3	16.4	17.1	13.8
Grain Size Analyses:	ASTM D422	No reporting limits				
Clay (%)		<0.005 mm	14.6	13.2	12.2	6.7
Silt (%)		>0.005-<0.074 mm	39.8	42.0	41.0	43.2
Fine Sand (%)		>0.074 -<0.417 mm	39.1	36.6	41.6	45.6
Medium Sand (%)		>0.417- <1.651 mm	5.9	4.2	4.9	4.3
Coarse Sand and Larger (%)		>1.651 mm	0.6	4.0	0.3	0.2
Moisture (%)			24.5	22.2	24.6	26.2
Nitrate + Nitrite (as N)	353.2	0.50	13.3	108	10.2	13.1
pH	9045	NA	7.52	7.09	5.86	4.40
Total Kjeldahl Nitrogen	351.2	3.12	396	206	233	597
Total Organic Carbon	9060	4000	31800	23300	26900	42400
Total Solids (%)	CLPILM03.0		75.5	76.3	75.7	74.0

^a All units are in mg/kg dry weight except for cation exchange capacity, grain size, and pH which are expressed as meq/100g, percent, and standard units, respectively.

Table A-2 Metals in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Aluminum (Al)	6010B	5	8300	1000	8700	5200
Antimony (Sb)	6020	0.250	0.6	0.5	0.4	1.0
Arsenic (As)	6020	0.25	3.6	4.0	3.5	2.1
Barium (Ba)	6020	0.250	67	160	55	14
Beryllium (Be)	6020	0.050	0.5	0.6	0.4	0.2
Cadmium (Cd)	6020	0.025	0.48	0.35	0.13	0.6
Calcium (Ca)	6010B	25	860	5400	1500	520
Chromium (Cr)	6020	0.100	13	11	8.2	5.9
Cobalt (Co)	6020	0.250	4.5	5.0	3.6	2.9
Copper (Cu)	6020	0.250	23	11	8.4	5.2
Iron (Fe)	6010B	5	8600	9700	7700	7600
Lead (Pb)	6020	0.250	93	110	25	28
Magnesium (Mg)	6010B	5	1100	1800	940	450
Manganese (Mn)	6010B	0.5	380	590	340	130
Mercury; Total (Hg ⁰)	^b	0.0001	10.086	2.685	0.149	0.076
Mercury; Monomethylmercury (CH ₃ Hg ⁺)	^b	0.00001	0.00518	0.00206	0.00099	0.00054
Nickel (Ni)	6020	0.250	9.4	9.3	6.6	5.2

Table A-2. Metals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Potassium (K)	6010B	5	470	460	420	320
Selenium (Se)	6020	0.25	0.4	0.4	0.3	<0.25
Silver (Ag)	6020	0.050	0.07	0.08	0.05	0.28
Sodium (Na)	6010B	25	390	500	400	420
Thallium (Tl)	6020	0.100	0.1	0.1	0.1	<0.1
Vanadium (V)	6020	0.250	17	19	14	15
Zinc (Zn)	6010B	1	160	210	59	34

^a All units are in mg/kg dry weight.

^b Total mercury and monomethylmercury analyzed via the procedures given in Brooks Rand, Ltd. (1998a, 1998b).

Table A-3. Priority Pollutant Volatile Organics in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Acrolein	8260B	13	<13	<13		>13
	8260B	15			<15	
Acrylonitrile	8260B	13	<13	<13		<13
	8260B	15			<15	
Benzene	8260B	1	<1	<1	<1	<1
Bromodichloromethane	8260B	1	<1	<1	<1	<1
Bromoform	8260B	1	<1	<1	<1	<1
Bromomethane	8260B	1	<1	<1	<1	<1
Carbon Tetrachloride	8260B	1	<1	<1	<1	<1
Chlorobenzene	8260B	1	<1		<1	<1
	8260B	6		<6		
Chloroethane	8260B	1	<1	<1	<1	<1
2-Chloroethylvinyl Ether	8260B	1	<1	<1	<1	<1
Chloroform	8260B	1	<1	<1	<1	<1
Chloromethane	8260B	1	<1	<1	<1	<1
Dibromochloromethane	8260B	1	<1	<1	<1	<1
1,1-Dichloroethane	8260B	1	<1	<1	<1	<1
1,1-Dichloroethene	8260B	1	<1	<1	<1	<1
1,2-Dichloroethane	8260B	1	<1	<1	<1	<1
trans-1,2-Dichloroethene	8260B	1	<1	<1	<1	<1

Table A-3. Priority Pollutant Volatile Organics in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
1,2-Dichloropropane	8260B	1	<1	<1	<1	<1
1,3-Dichloropropane	8260B	1	<1	<1	<1	<1
cis-1,3-Dichloropropene	8260B	1	<1	<1	<1	<1
trans-1,3-Dichloropropene	8260B	1	<1	<1	<1	<1
Ethylbenzene	8260B	1	<1	<1	<1	<1
Methylene Chloride	8260B	1	<1	<1	<1	<1
Tetrachloroethene	8260B	1	<1	<1	<1	<1
1,1,2,2-Tetrachloroethane	8260B	1	<1	<1	<1	<1
Toluene	8260B	1	<1	<1	<1	<1
Trichloroethene	8260B	1	<1	<1	<1	<1
1,1,1-Trichloroethane	8260B	1	<1	<1	<1	<1
1,1,2-Trichloroethane	8260B	1	<1	<1	<1	<1
Trichlorofluoromethane	8260B	1	<1	<1	<1	<1
Vinyl Chloride	8260B	1	<1	<1	<1	<1
Total Xylene	8260B	1				<1
	8260B	2	<2	<2	<2	

^a All units are in mg/kg dry weight.

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Acenaphthene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Acenaphthylene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Anthracene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Benzidine	8270C	2.2	<2.2	<2.2		
	8270C	2.4				<2.4
	8270C	2.6			<2.6	
Benzo(a) Anthracene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8070C	0.50			<0.50	
Benzo(b) Fluoranthene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Benzo(k) Fluoranthene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Benzo(g,h,i) Perylene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Benzo(a) Pyrene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Bis(2-Chloroethoxy) Methane	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Bis(2-Chloroethyl) Ether	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Bis(2-Chloroisopropyl) Ether	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Bis(2-Ethylhexyl) Phthalate	8270C	0.43	1.5	0.48		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
4-Bromophenyl Phenyl Ether	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Butyl Benzyl Phthalate	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2-Chloronaphthalene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
4-Chlorophenyl Phenyl Ether	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Chrysene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Dibenzo(a,h) Anthracene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
1,2-Dichlorobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
1,3-Dichlorobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
1,4-Dichlorobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
3,3'-Dichlorobenzidine	8270C	0.87	<0.87	<0.87		
	8270C	0.94				<0.94
	8270C	1.0			<1.0	
Dimethyl Phthalate	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Diethyl Phthalate	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Di-n-Butyl Phthalate	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Di-n-Octyl Phthalate	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2,4-Dinitrotoluene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2,6-Dinitrotoluene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Fluoranthene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Fluorene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Hexachlorobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Hexachloroethane	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Hexachlorobutadiene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Hexachlorocyclopentadiene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Indeno(1,2,3-cd) Pyrene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Isophorone	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Naphthalene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Nitrobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
N-Nitrosodimethylamine	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
N-Nitrosodiphenylamine	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
N-Nitrosodi-n-propylamine	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

Table A-4. Priority Pollutant Base Neutrals in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Phenanthrene	8270C	0.43	<0.43			
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
Pyrene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
1,2,4-Trichlorobenzene	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

^a All units are in mg/kg dry weight.

Table A-5. Priority Pollutant Acid Extractables in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
4-Chloro-3-Methyl Phenol	8270C	0.87	<0.87	<0.87		
	8270C	0.94				<0.94
	8270C	1.0			<1.0	
2-Chlorophenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2,4-Dichlorophenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2,4-Dimethylphenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
4,6-Dinitro-2-Methyl Phenol	8270C	2.2	<2.2	<2.2		
	8270C	2.4				<2.4
	8270C	2.6			<2.6	
2,4-Dinitrophenol	8270C	2.2	<2.2	<2.2		
	8270C	2.4				<2.4
	8270C	2.6			<2.6	

Table A-5. Priority Pollutant Acid Extractables in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
2-Nitrophenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
4-Nitrophenol	8270C	2.2	<2.2	<2.2		
	8270C	2.4				<2.4
	8270C	2.6			<2.6	
Pentachlorophenol	8270C	2.2	<2.2	<2.2		
	8270C	2.4				<2.4
	8270C	2.6			<2.6	
Phenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	
2,4,6-Trichlorophenol	8270C	0.43	<0.43	<0.43		
	8270C	0.46				<0.46
	8270C	0.50			<0.50	

^a All units are in mg/kg dry weight.

Table A-6. Priority Pollutant Organophosphorus Pesticides in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Azinphos-methyl (Guthion)	8140	50	ND	ND	ND	ND
Baytex (Fenthion)	8140	50	ND	ND	ND	ND
Bolstar	8140	50	ND	ND	ND	ND
Coumaphos	8140	50	ND	ND	ND	ND
Demeton O&S	8140	50	ND	ND	ND	ND
Diazinon	8140	50	ND	ND	ND	ND
Dichlorvos	8140	50	ND	ND	ND	ND
Disyston (Disulfoton)	8140	50	ND	ND	ND	ND
Dursban (Chlorpyrifos)	8140	50	ND	ND	ND	ND
Ethoprop	8140	50	ND	ND	ND	ND
Merphos	8140	50	ND	ND	ND	ND
Methyl parathion	8140	50	ND	ND	ND	ND
Phorate (Thimet)	8140	50	ND	ND	ND	ND
Ronnel	8140	50	ND	ND	ND	ND
Tetrachlorvinophos	8140	50	ND	ND	ND	ND
Tokuthion (Propiophos)	8140	50	ND	ND	ND	ND
Trichloronate	8140	50	ND	ND	ND	ND

^a All units are in mg/kg dry weight.

Table A-7. Chlorinated Pesticides and Herbicides in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
<u>Pesticides:</u>						
Aldrin	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
α-BHC	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
β-BHC	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
γ-BHC (Lindane)	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
δ-BHC	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
4,4'-DDD	8081A/8082	0.4	<0.4	<0.4	<0.4	<0.4
4,4'-DDE	8081A/8082	0.1	<0.1	<0.1		<0.1
	8081A/8082	0.2			<0.2	
4,4'-DDT	8081A/8082	0.4	<0.4	<0.4	<0.4	<0.4
Dieldrin	8081A/8082	0.1	<0.1	<0.1		<0.1
	8081A/8082	0.2			<0.2	
Endosulfan I	8081A/8082	0.1	<0.1	<0.1		<0.1
	8081A/8082	0.2			<0.2	

Table A-7. Chlorinated Pesticides And Herbicides in the Graces Quarters Study Soils (Continued)

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
Endosulfan II	8081A/8082	0.4	<0.4	<0.4	<0.4	<0.4
Endosulfan Sulfate	8081A/8082	0.4	<0.4	<0.4	<0.4	<0.4
Endrin	8081A/8082	0.1	<0.1	<0.1		<0.1
	8081A/8082	0.2			<0.2	
Endrin Aldehyde	8081A/8082	0.4	<0.4	<0.4	<0.4	<0.4
Heptachlor	8081A/8082	0.07	<0.07	<0.07	<0.07	<0.07
	8081A/8082	0.08			<0.08	
Heptachlor Epoxide	8081A/8082	0.07	<0.07	<0.07		<0.07
	8081A/8082	0.08			<0.08	
Technical Chlordane	8081A/8082	1	<1	<1		<1
	8081A/8082	2			<2	
Total PCBs	8081A/8082	1	<1	<1		<1
	8081A/8082	2			<2	
Toxaphene	8081A/8082	4	<4	<4	<4	<4
<u>Herbicides:</u>						
2,4-D	8151A	0.007	<0.07	<0.07		<0.07
	8151A	0.08			<0.08	
2,4,5-TP (Silvex)	8151A	0.03	<0.03	<0.03	<0.03	<0.03

^a All units are in mg/kg dry weight.

Table A-8. Explosives in the Graces Quarters Study Soils^a

Analyte	EPA Method	Reporting Limits	TQ1245D (High)	TQ1227 (Int.)	TQ1246 (Low)	On Site Reference
2-Amino-4,6-dinitrotoluene (2-AM-4,6 DNT)	b	0.05	BDL	BDL	BDL	BDL
4-Amino-2,6-dinitrotoluene (4-AM-2,6-DNT)	b	0.05	BDL	BDL	BDL	BDL
1,3-Dinitrobenzene (1,3-DNB)	b	0.05	BDL	BDL	BDL	BDL
2,4-Dinitrotoluene (2,4-DNT)	b	0.05	BDL	BDL	BDL	BDL
2,6-Dinitro-toluene (2,6-DNT)	b	0.05	BDL	BDL	BDL	BDL
Hexahydro-1,3,5-trinitro-1,3,4-triazine (RDX)	b	0.05	BDL	BDL	BDL	BDL
N,2,4,6-tetranitro-N-methylaniline (tetryl)	b	0.05	BDL	BDL	BDL	BDL
Nitrobenzene (NB)	b	0.05	BDL	BDL	BDL	BDL
2-Nitrotoluene (2-NT)	b	0.05	BDL	BDL	BDL	BDL
3-Nitrotoluene (3-NT)	b	0.05	BDL	BDL	BDL	BDL
4-Nitrotoluene (4-NT)	b	0.05	BDL	BDL	BDL	BDL
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	b	0.05	BDL	BDL	BDL	BDL
1,3,5-Trinitrobenzene (TNB)	b	0.05	BDL	BDL	BDL	BDL
Trinitrotoluene (TNT)	b	0.05	BDL	BDL	BDL	BDL

^a All units are in mg/kg dry weight.

^c Explosives analyzed via the procedures given in USACEHR (1993).

APPENDIX B

GENERAL CHEMISTRY AND MERCURY IN THE SOIL DURING UPTAKE AND DEPURATION

Table B-1. General Chemistry And Mercury In the Soil During Uptake and Depuration - High Mercury Soil (TQ1245D)

Day	Rep	Temp (°C)	Moisture (%)	pH	T-Hg (ng/g dry wt.)	MMHg (ng/g dry wt.)
0	1	22.0	42	5.72	10129	5.79
	2				9735	5.64
	3				11313	4.15
	4				9168	5.12
4	1	21.8	43			
7	1	22.0	45			
12	1	22.0	46			
15	1	21.9	47			
19	1	21.9	45			
22	1	22.0	47			
26	1	21.8	45			
28	1				21791	8.53
	2				9427	11.00
	3				10092	8.73
	4				10679	9.86
29	1	21.8	49	5.93		
36	1	22.0	50			
40	1	21.9	52			
43	1	21.8	51			
47	1	21.9	52			
50	1	21.8	49			
54	1	21.8	48			
56	1	21.8	47			
Mean		21.9	47		11542	7.35
Min		21.8	42	5.72	9168	4.15
Max		22.0	52	5.93	21791	11.00

Table B-2. General Chemistry And Mercury In the Soil During Uptake and Depuration - Intermediate Mercury Soil (TQ1227)

Day	Rep	Temp (°C)	Moisture (%)	pH	T-Hg (ng/g dry wt.)	MMHg (ng/g dry wt.)
0	1	22.0	43	5.53	2721	2.35
	2				2581	2.45
	3				2845	1.26
	4				2592	2.17
4	1	21.9	45			
7	1	22.0	42			
12	1	22.0	44			
15	1	21.9	45			
19	1	21.9	47			
22	1	22.0	46			
26	1	21.8	47			
28	1				2898	2.82
	2				2843	3.27
	3				3611	2.64
	4				2506	3.49
29	1	21.8	49	6.00		
36	1	21.9	49			
40	1	21.8	51			
43	1	21.8	51			
47	1	22.0	48			
50	1	21.8	51			
54	1	21.8	50			
56	1	21.8	48			
Mean		21.9	47		2825	2.56
Min		21.8	42	5.53	2506	1.26
Max		22.0	51	6.00	3611	3.49

Table B-3. General Chemistry And Mercury In the Soil During Uptake and Depuration - Low Mercury Soil (TQ1246)

Day	Rep	Temp (°C)	Moisture (%)	pH	T-Hg (ng/g dry wt.)	MMHg (ng/g dry wt.)
0	1	22.0	43	5.70	151	1.27
	2				157	1.20
	3				144	1.29
	4				145	0.21
4	1	21.8	44			
7	1	22.0	47			
12	1	22.0	44			
15	1	21.9	47			
19	1	21.9	47			
22	1	22.0	48			
26	1	21.8	48			
28	1				177	1.75
	2				158	2.06
	3				162	1.65
	4				151	2.41
29	1	21.9	50	5.92		
36	1	22.0	51			
40	1	21.9	50			
43	1	21.8	50			
47	1	22.0	50			
50	1	21.9	48			
54	1	21.8	48			
56	1	21.9	47			
Mean		21.9	48		156	1.48
Min		21.8	43	5.70	144	0.21
Max		22.0	51	5.92	177	2.41

Table B-4. General Chemistry And Mercury In the Soil During Uptake and Depuration - Reference Soil

Day	Rep	Temp (°C)	Moisture (%)	pH	T-Hg (ng/g dry wt.)	MMHg (ng/g dry wt.)
0	1	22.0	44	5.50	73.4	0.637
	2				61.1	0.492
	3				87.4	0.519
	4				81.9	0.508
4	1	21.8	47			
7	1	22.0	44			
12	1	22.0	45			
14	1	21.9	48			
18	1	21.9	44			
21	1	22.0	46			
25	1	21.8	46			
28	1	21.8	48		109	0.654
	2				94.8	2.46
	3				79.1	1.31
	4				90.1	2.34
35	1	21.9	50	5.98		
39	1	21.9	49			
42	1	21.8	51			
46	1	22.2	49			
49	1	21.9	50			
53	1	21.9	48			
56	1	21.9	48			
Mean		21.9	47		85	1.12
Min		21.8	44	5.50	61	0.49
Max		22.2	51	5.98	109	2.46

APPENDIX C

SURVIVAL, GROWTH, AND MERCURY IN THE EARTHWORM DURING UPTAKE AND DEPURATION

Table C-1. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - High Mercury Soil (TQ1245D): Uptake Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
0	1	0	29.304	44.1	8.8
	2	0	21.958	63.0	4.1
	3	0	27.406	34.6	6.9
	4	0	20.286	52.6	1.7
1	1	0	25.421	1073	5.7
	2	0	21.430	794	31.8
	3	0	22.819	471	25.6
	4	0	21.958	321	23.9
2	1	0	22.526	456	28.5
	2	0	17.195	1434	35.1
	3	0	24.932	819	27.7
	4	0	24.267	743	34.2
4	1	0	20.080	213	45.9
	2	0	20.550	778	38.3
	3	0	20.472	978	41.1
	4	0	27.494	895	38.8
7	1	0	20.208	2735	118
	2	0	21.049	867	71.6
	3	2	28.634	4000	100
	4	0	23.484	1598	115
14	1	1	26.115	2759	125
	2	0	25.010	1746	96.8
	3	0	26.409	4810	291
	4	0	20.873	5008	146
21	1	0	31.583	4977	414
	2	1	20.671	3672	267
	3	1	33.266	6687	218
	4	3	21.854	4706	298
28	1	0	34.654	14875	981
	2	0	33.158	6164	361
	3	0	34.537	14397	714
	4	0	32.551	19063	867

Table C-1. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - High Mercury Soil (TQ1245D): Depuration Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
35	1	0	31.358	7616	360
	2	0	28.854	3902	256
	3	1	28.952	3874	366
	4	1	29.799	2441	265
42	1	2	34.282	3891	363
	2	2	35.786	2338	257
	3	2	31.446	4374	321
	4	2	28.842	1477	303
49	1	0	43.525	1577	401
	2	3	33.088	1075	199
	3	2	34.209	3104	268
	4	0	40.513	6158	413
56	1	1	47.384	2975	591
	2	0	38.596	3553	483
	3	1	53.567	3282	413
	4	1	48.199	1649	343

Table C-2. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - Intermediate Mercury Soil (TQ1227): Uptake Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
0	1	0	29.304	44.1	8.8
	2	0	21.958	63.0	4.1
	3	0	27.406	34.6	6.9
	4	0	20.286	52.6	1.7
1	1	0	25.724	219	3.9
	2	0	22.398	249	0.8
	3	0	23.484	121	<12
	4	0	19.513	149	<14
2	1	0	26.986	226	15.4
	2	0	22.702	234	22.1
	3	0	22.623	275	21.9
	4	0	16.549	182	24.7
4	1	0	29.627	328	23.0
	2	0	28.228	321	25.0
	3	0	24.462	361	33.8
	4	0	21.401	292	24.1
7	1	0	27.700	321	45.0
	2	0	26.595	356	47.0
	3	0	23.650	602	61.0
	4	0	27.289	256	49.0
14	1	0	25.763	756	63.1
	2	0	32.884	739	64.0
	3	0	28.619	924	60.4
	4	0	29.343	759	59.2
21	1	0	36.610	880	101
	2	0	33.383	1040	133
	3	0	28.394	866	96.6
	4	0	29.294	1238	126
28	1	0	36.865	1033	191
	2	0	31.055	1525	252
	3	0	31.377	2074	249
	4	0	33.764	1195	318

Table C-2. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - Intermediate Mercury Soil (TQ1227): Depuration Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
35	1	0	30.321	788	206
	2	0	31.025	791	184
	3	0	30.057	726	199
	4	0	33.275	977	198
42	1	1	29.180	691	195
	2	1	40.254	517	126
	3	1	28.517	385	178
	4	0	40.435	769	199
49	1	1	39.885	573	201
	2	0	43.584	781	214
	3	0	41.393	729	244
	4	1	42.895	581	221
56	1	0	51.996	625	406
	2	1	44.015	760	364
	3	0	46.235	969	571
	4	0	39.818	658	262

Table C-3. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - Low Mercury Soil (TQ1246): Uptake Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
0	1	0	29.304	44.1	8.8
	2	0	21.958	63.0	4.1
	3	0	27.406	34.6	6.9
	4	0	20.286	52.6	1.7
1	1	0	19.210	68.0	10.4
	2	0	25.401	61.8	9.8
	3	0	21.802	82.4	11.3
	4	0	22.526	92.7	9.9
2	1	0	22.790	78.7	16.4
	2	0	17.361	88.6	22.3
	3	0	23.191	88.5	17.5
	4	0	21.117	90.7	15.5
4	1	0	26.526	97.9	25.0
	2	0	17.469	122	20.7
	3	0	26.418	140	21.2
	4	0	30.703	111	24.2
7	1	0	21.313	144	61.2
	2	0	18.418	145	59.5
	3	0	20.677	128	53.3
	4	0	19.024	121	45.2
14	1	0	27.387	220	105
	2	0	26.223	175	73.7
	3	0	23.641	227	101
	4	0	24.453	196	97.4
21	1	0	35.798	276	136
	2	0	35.896	248	106
	3	0	29.724	217	143
	4	0	40.738	299	153
28	1	0	41.481	323	164
	2	1	31.125	320	220
	3	0	37.236	302	151
	4	0	32.541	387	179

Table C-3. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - Low Mercury Soil (TQ1246): Depuration Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
35	1	0	33.050	316	138
	2	0	30.712	352	89
	3	1	36.940	258	128
	4	0	35.808	263	162
42	1	1	34.483	343	195
	2	0	33.285	214	69.6
	3	1	43.178	293	96.6
	4	1	37.961	301	119
49	1	0	50.832	296	206
	2	0	40.738	247	214
	3	0	53.923	303	232
	4	1	47.253	303	236
56	1	0	45.022	486	302
	2	0	56.788	322	266
	3	1	63.566	386	298
	4	0	48.259	437	300

**Table C-4. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration -
Reference Soil: Uptake Phase**

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
0	1	0	29.304	44.1	8.8
	2	0	21.958	63.0	4.1
	3	0	27.406	34.6	6.9
	4	0	20.286	52.6	1.7
1	1	0	17.977	91.1	13.7
	2	0	31.260	95.3	3.5
	3	0	24.746	120	5.6
	4	0	25.235	62.7	8.6
2	1	0	24.257	71.6	20.4
	2	0	23.562	74.0	19.6
	3	0	23.543	75.0	16.1
	4	0	31.456	73.1	8.3
4	1	0	21.616	98.0	20.9
	2	0	29.333	87.1	10.0
	3	0	26.820	80.5	19.3
	4	0	22.487	69.2	20.9
7	1	0	27.279	106	38.2
	2	0	23.553	107	43.9
	3	0	21.812	150	37.4
	4	0	20.442	136	64.9
14	1	0	23.787	145	46.8
	2	0	32.874	167	30.7
	3	0	26.223	127	16.7
	4	0	28.433	145	40.9
21	1	0	31.945	214	95.8
	2	0	31.201	281	169
	3	0	31.006	164	79.1
	4	0	27.054	292	162
28	1	0	41.824	204	52.7
	2	0	37.804	288	71.7
	3	0	35.926	292	148
	4	0	38.928	283	151

Table C-4. Survival, Growth, and Mercury in the Earthworm During Uptake and Depuration - Reference Soil: Depuration Phase

Day	Rep	No. Dead	Dry Weight per Worm (mg)	T-Hg in Worm (ng/g dry wt.)	MMHg in Worm (ng/g dry wt.)
35	1	0	33.148	262	145
	2	0	35.955	251	94.6
	3	0	35.857	291	196
	4	0	34.243	334	113
42	1	0	44.768	274	128
	2	0	39.838	252	144
	3	0	39.144	292	164
	4	0	34.351	242	126
49	1	1	50.307	Lost	Lost
	2	1	40.635	365	252
	3	0	38.351	270	156
	4	0	48.651	303	198
56	1	0	55.282	205	311
	2	0	52.475	345	236
	3	0	51.487	272	337
	4	0	52.553	413	252

APPENDIX D

STATISTICS FOR THE GROWTH ANALYSES DURING UPTAKE AND DEPURATION

Table D-1. Earthworm Growth During Study- Analysis of Covariance Run 1

Source	DF	Type III Sum of Squares	Mean Square	F-Value	Pr > F
Concentration	3	74.0279	24.6760	1.54	0.0208
Phase	1	237.9661	237.9661	14.86	0.0002
Concentration *Phase	3	92.1896	30.7299	1.92	0.1307
Pday	1	3965.9378	3965.9378	247.59	0.0001
Pday *Concentration	3	151.5481	50.5160	3.15	0.0277
Pday *Phase	1	131.6048	131.6048	8.22	0.0050
Pday *Concentration *Phase	3	19.9186	6.6395	0.41	0.7429

Table D-2. Earthworm Growth During Study- Analysis of Covariance Run 2

Source	DF	Type III Sum of Squares	Mean Square	F-Value	Pr > F
Concentration	3	74.0279	24.6760	1.46	0.2304
Phase	1	237.9661	237.9661	14.03	0.0003
Pday	1	3965.9378	3965.9378	233.89	0.0001
Pday *Concentration	3	151.5481	50.5160	2.98	0.0343
Pday *Phase	1	131.6048	131.6048	7.76	0.0062

APPENDIX E

STATISTICS FOR THE MODEL ANALYSES OF THE TOTAL MERCURY UPTAKE AND DEPURATION TREATMENT CURVES

Table E-1. Rate of Total Mercury Uptake in All Treatments in the Uptake Phase

Concentration	Slope Estimate	T for HO: Slope=0	Pr > T	Std. Error of Estimate
Reference	0.0556	8.03	0.0001	0.0069
Low	0.0579	8.38	0.0001	0.0069
Intermediate	0.0823	11.91	0.0001	0.0069
High	0.1178	17.04	0.0001	0.0069

Table E-2. Comparison of Total Mercury Slopes for Each Treatment to the Reference Treatment In the Uptake Phase

Treatment Comparison	Estimated Difference	T for HO: Equal Slopes	Pr > T	Std. Error of Difference
Low vs Reference	0.0024	0.27	0.7895	0.0090
Intermediate vs Reference	0.0268	2.99	0.0035	0.0090
High vs Reference	0.0623	6.93	0.0001	0.0090

Table E-3. Mean Total Mercury Concentrations at Day 28 of Uptake Phase

Treatment	Mean log T-Hg at Day 28	Mean Minus Reference	T for HO: Equal Reference	Pr > T
Reference	5.5252 (251) ^a			
Low	5.7040 (300)	0.1789	1.02	0.3106
Intermediate	7.2782 (1,448)	1.7531	9.99	0.0001
High	9.3098 (11,046)	3.7846	21.56	0.0001

^a Data in parentheses are mean arithmetic T-Hg concentrations in ng/g dry weight.

Table E-4. Rate of Total Mercury Decrease in All Treatments in the Depuration Phase

Concentration	Slope Estimate	T for HO: Slope=0	Pr > T	Std. Error of Estimate
Reference	0.0043	0.57	0.5723	0.0075
Low	0.0068	0.92	0.3588	0.0074
Intermediate	0.0213	-2.87	0.0054	0.0074
High	0.0512	-6.91	0.0001	0.0074

Table E-5. Comparison of Total Mercury Slopes for Each Treatment to the Reference Treatment in the Depuration Phase

Treatment Comparison	Estimated Difference	T for HO: Equal Slopes	Pr > T	Std. Error of Difference
Low vs Reference	0.0022	0.23	0.8212	0.0098
Intermediate vs Reference	0.0259	-2.64	0.0104	0.0098
High vs Reference	0.0558	-5.68	0.0001	0.0198

Table E-6. Predicted Mean Total Mercury Concentrations at Day 56 of the Depuration Phase

Treatment	Mean log T-Hg at Day 56	Mean Minus Reference	T for HO: Equal Reference	Pr > T
Reference	5.7124 (303) ^a			
Low	5.9423 (381)	0.2299	1.13	0.2644
Intermediate	6.6428 (767)	0.9304	4.56	0.0001
High	7.9559 (2,852)	2.2435	10.98	0.0001

^a Data in parentheses are mean arithmetic T-Hg concentrations in ng/g dry weight tissue.

APPENDIX F

STATISTICS FOR THE MODEL ANALYSES OF THE MONOMETHYLMERCURY UPTAKE AND DEPURATION TREATMENT CURVES

Table F-1. Rate of Monomethylmercury Uptake in All Treatments in the Uptake Phase

Concentration	Slope Estimate	T for HO: Slope=0	Pr > T	Std. Error of Estimate
Reference	0.1097	10.24	0.0001	0.0107
Low	0.1199	11.19	0.0001	0.0107
Intermediate	0.1386	12.93	0.0001	0.0107
High	0.1431	13.36	0.0001	0.0107

Table F-2. Comparison of Monomethylmercury Slopes for Each Treatment to the Reference Treatment In the Uptake Phase

Treatment Comparison	Estimated Difference	T for HO: Equal Slopes	Pr > T	Std. Error of Difference
Low vs Reference	0.0102	0.73	0.4674	0.0139
Intermediate vs Reference	0.0288	2.07	0.0412	0.0139
High vs Reference	0.0334	2.40	0.0184	0.0139

Table F-3. Predicted Mean Monomethylmercury Concentrations at Day 28 of Uptake Phase

Treatment	Mean log T-Hg at Day 28	Mean Minus Reference	T for HO: Equal Reference	Pr > T
Reference	4.5941 (99) ^a			
Low	5.1723 (176)	0.5781	2.12	0.0360
Intermediate	5.3264 (206)	0.7322	2.69	0.0083
High	6.2725 (530)	1.6783	6.17	0.0001

^a Data in parentheses are mean arithmetic MMHg concentrations in ng/g dry weight tissue.

Table F-4. Rate of Monomethylmercury Change in All Treatments in the Depuration Phase

Concentration	Slope Estimate	T for HO: Slope=0	Pr > T	Std. Error of Estimate
Reference	0.0365	6.01	0.0001	0.0061
Low	0.0223	3.72	0.0004	0.0060
Intermediate	0.0141	2.36	0.0214	0.0060
High	-0.0121	-2.02	0.0474	0.0060

Table F-5. Comparison of Monomethylmercury Slopes for Each Treatment to the Reference Treatment In the Depuration Phase

Treatment Comparison	Estimated Difference	T for HO: Equal Slopes	Pr > T	Std. Error of Difference
Low vs Reference	-0.0143	-1.67	0.0999	0.0085
Intermediate vs Reference	-0.0224	-2.62	0.0108	0.0085
High vs Reference	-0.0487	-5.70	0.0001	0.0085

Table F-6. Predicted Mean Monomethylmercury Concentrations at Day 56 of the Depuration Phase

Treatment	Mean log MMHg at Day 56	Mean Minus Reference	T for HO: Equal Reference	Pr > T
Reference	5.6244 (277) ^a			
Low	5.7621 (318)	0.1377	0.78	0.4409
Intermediate	5.9378 (379)	0.3133	1.76	0.0823
High	6.1301 (459)	0.5057	2.85	0.0059

^a Data in parentheses are mean arithmetic MMHg concentrations in ng/g dry weight tissue.